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INFLUENCE OF ENVIRONMENT DURING MATURATION ON THE DISEASE REACTION AND YIELD OF WHEAT AND BARLEY[1]

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INTRODUCTION

The influence of size of seed on development and yield of plants has been studied by numerous workers. Little attention has been given however, to the influence, on the subsequent crop, of other characteristics of the seed determined by the environment while the seed was attached to the mother plant, or to the effect of seed characteristics on other aspects of the crop besides yield. The experiments reported herein were undertaken to study reaction to seedling blight caused by *Gibberella saubinetii* (Mont.) Sacc.; reaction to bunt caused by *Tilletia levis* Kühn; and yield from seed produced in different environments. For this purpose seed of varieties of wheat and barley, produced at a number of places in the United States and Canada, was tested for reaction to seedling blight in the greenhouse at Madison, Wis., and for reaction to bunt and for yield in field trials at Moccasin, Mont., Madison, Wis., and Aberdeen, Idaho. Although the primary purpose of the experiments was to determine the effect of different environments on disease reaction and yield of seed lots of the same variety, data were also obtained on the relative resistance of the varieties to seedling blight.

REVIEW OF LITERATURE

Some original data and a review of the literature dealing with the subject of physiologic predetermination have been presented by Kidd and West (*8*, *9*).[3] They conclude, largely on the basis of the literature, that (1) larger seeds give rise to more vigorous plants and a better yield, and that (2) the environment under which seed is produced may have a marked influence on the development of plants of the next generation. Their evidence consists chiefly of the effects of size of seed.

Seed of certain spring wheat varieties produced in central Illinois was found by Dickson et al. (*5*) to be more susceptible to *Gibberella saubinetii* than seed of the same varieties produced at Madison, Wis. Similar differences were found with late-maturing types of corn,

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[3] Reference is made by number (italic) to Literature Cited, p. 748.

except that seed produced at Madison was the more susceptible. These workers report small differences in the composition of the endosperm but marked differences in the composition of the embryo. Hoppe et al. (*7*) also found that the environment during the period of growth and maturation of the mother plant greatly affects the susceptibility of corn seedlings to *G. saubinetii*. They harvested self-pollinated corn at intervals and found that the degree of maturity of the seed influenced the disease reaction and yield of the subsequent crop.

Many workers have studied the relation of size of seed to germination, growth of the plant, and yield. In all the experiments, however, seed of the different sizes has been obtained by separations made with screens or a fanning mill, and not by producing the seed under different environmental conditions. Kiesselbach and Helm (*10*) have presented a comprehensive review of the literature dealing with this subject, and it seems unnecessary to present another review at this time. They also conducted an elaborate series of experiments, and their conclusions summarize the information on the effect of size of seed on yield, as follows (*10, p. 69*):

> (1) When space-planted to permit maximum development, a higher individual plant yield is obtained from large than from small seeds. * * *
> (2) When planted in equal numbers at a rate optimum for large seed, a lower yield is obtained from the small than from the large seed. * * *
> (3) When planted in equal weights, at a rate optimum for the large seed, all three grades—large, small, and unselected—yield equally.

Arny and Garber (*1*) made a critical study of the relation of seed size to rate of growth of spaced plants and yield in wheat. They found a positive correlation between size of seed and size of plants. The maximum difference in size of plants occurred at 6 weeks of age, with a tapering off of the difference as the plants approached maturity. They also found a positive correlation between size of seed and yield, the relation being more pronounced on poor than on fertile soil.

Kucheryaeva (*11*) found that immature seed, harvested when the kernels weighed 10 g per 1,000, yielded the same in a favorable year as mature seed from the same crop that weighed 29 g per 1,000 kernels. Differences in favor of the larger seed were obtained, however, in dry, unfavorable years.

MATERIAL AND METHODS

Four varieties of spring wheat (*Triticum aestivum* L., syn. *T. vulgare* Vill.) and three of barley (*Hordeum vulgare* L.) were chosen for the present study and were grown at 10 different locations in the United States and Canada in 1929 and at 12 locations in 1930. The wheat varieties were Marquis (C. I.[4] 3641), Hard Federation (C. I. 4733), Peliss selection 89, and Illinois No. 1. The barley varieties were Trebi (C. I. 936), Hannchen selection 1 (C. I. 5462), and Oderbrucker (Wisconsin Pedigree No. 5) (C. I. 1272). All but Illinois No. 1 wheat had been carefully selected and the seed used was reasonably pure. The Illinois No. 1 was an unselected type and was found to be mixed. Illinois No. 1A, a mass-selected strain, was substituted for it in 1930.

[4] C. I. refers to accession number of the Division of Cereal Crops and Diseases (formerly Office of Cereal Investigations).

The 10 locations at which seed was produced in 1929 and the 12 at which it was produced in 1930, together with yield of the crop, weight of 1,000 kernels, and protein content of the grain from each, are given in tables 1 and 2. Graphs showing the weight per 1,000 kernels and the protein content are presented later (see figs. 1, 2, 4, 5, and 6) in connection with other data. Seed was sent to each station for sowing each year to insure use of the same seed at all stations. Each variety was grown in three nursery plots of three rows each, the center row only being harvested for yield. All seedings were made at the normal rate and date at each station.

TABLE 1.—*Yield and protein content of the grain and weight of 1,000 kernels of 4 varieties of wheat and 3 of barley, grown at 10 experiment stations in the United States and Canada in 1929*

WHEAT VARIETIES

Station where grown	Marquis			Illinois No. 1			Hard Federation			Peliss selection 89		
	Acre yield	Protein in grain	Weight of 1,000 kernels	Acre yield	Protein in grain	Weight of 1,000 kernels	Acre yield	Protein in grain	Weight of 1,000 kernels	Acre yield	Protein in grain	Weight of 1,000 kernels
	Bu.	*Pct.*	*G*	*Bu.*	*Pct.*	*G*	*Bu.*	*Pct.*	*G*	*Bu.*	*Pct.*	*G*
Aberdeen, Idaho	41.5	11.4	35.1	38.0	11.8	34.0	42.8	11.1	39.8	43.3	11.0	61.1
Bozeman, Mont	37.4	16.7	34.8	31.0	16.3	30.5	45.6	13.6	35.6	60.9	14.6	60.6
Felt, Idaho	17.2	15.6	32.8	21.3	14.0	28.6	24.1	16.5	35.6	26.8	11.1	55.2
Hays, Kans	5.1	17.6	16.6	6.7	13.4	17.4	10.3	16.7	18.2	12.8	18.6	27.0
Indian Head, Saskatchewan	34.1	15.3	29.0	31.3	17.1	28.5	29.6	14.4	34.8	50.3	15.1	52.4
Madison, Wis	2.9	13.9	14.8	2.7	12.9	15.8	1.7	12.8	15.2	.3	.....	30.2
Mandan, N. Dak	5.7	15.4	19.2	4.9	14.2	20.0	4.9	14.8	23.0	7.1	15.8	36.3
Moccasin, Mont	8.2	18.2	18.0	9.7	19.1	18.4	7.6	16.9	24.2	10.8	17.5	29.7
Moro, Oreg	13.2	14.9	20.2	11.7	15.2	24.8	18.3	13.6	28.9	15.9	14.6	37.0
North Platte, Nebr	17.8	16.8	19.4	14.1	16.1	19.0	15.2	14.5	18.8	21.6	16.1	39.1

BARLEY VARIETIES

	Hannchen selection 1			Trebi			Oderbrucker		
	Acre yield	Protein in grain	Weight of 1,000 kernels	Acre yield	Protein in grain	Weight of 1,000 kernels	Acre yield	Protein in grain	Weight of 1,000 kernels
	Bushels	*Percent*	*Grams*	*Bushels*	*Percent*	*Grams*	*Bushels*	*Percent*	*Grams*
Aberdeen, Idaho	63.4	12.1	42.8	67.1	11.8	48.1	28.6	13.2	37.2
Bozeman, Mont	63.4	13.3	38.3	68.5	11.9	44.9	42.9	13.3	37.2
Felt, Idaho	37.3	14.3	37.7	30.0	12.1	44.2	11.5	14.2	33.7
Hays, Kans	23.6	15.5	17.2	35.1	15.2	22.6	16.5	14.6	16.3
Indian Head, Saskatchewan	50.4	13.3	31.2	56.0	12.2	34.7	33.3	13.6	26.0
Madison, Wis	19.3	12.4	29.4	8.9	12.3	36.5	9.4	11.4	26.1
Mandan, N. Dak	6.7	13.3	26.3	5.7	13.0	30.6	3.2	14.0	25.7
Moccasin, Mont	15.3	17.2	23.2	17.1	12.1	26.2	2.0	17.7	19.5
Moro, Oreg	24.1	12.3	24.9	23.9	12.8	27.1	4.4	13.5	20.2
North Platte, Nebr	31.3	13.7	19.2	30.0	13.4	26.5	18.0	14.9	20.3

TABLE 2.—*Yield and protein content of the grain and weight of 1,000 kernels of 4 varieties of wheat and 3 of barley, grown at 12 experiment stations in the United States and Canada in 1930*

WHEAT VARIETIES

Station where grown	Marquis			Illinois No. 1A			Hard Federation			Peliss selection 89		
	Acre yield	Protein in grain	Weight of 1,000 kernels	Acre yield	Protein in grain	Weight of 1,000 kernels	Acre yield	Protein in grain	Weight of 1,000 kernels	Acre yield	Protein in grain	Weight of 1,000 kernels
	Bu.	*Pct.*	*G.*	*Bu.*	*Pct.*	*G.*	*Bu.*	*Pct.*	*G.*	*Bu.*	*Pct.*	*G.*
Aberdeen, Idaho	62.0	12.2	35.3	64.9	12.8	32.7	60.8	12.0	40.3	65.6	11.3	58.3
Arlington, Va	2.3	12.9	22.0	4.5	13.9	22.8	2.6	12.9	16.5	3.5	13.9	30.3
Bozeman, Mont	65.4	14.1	37.3	61.9	15.0	32.8	44.9	13.6	39.2	81.5	13.0	56.0
Felt, Idaho	17.0	14.8	27.4	14.7	16.2	27.1	9.2	15.8	31.1	20.7	14.4	47.1
Hays, Kans	6.3	15.6	13.4	5.3	15.5	13.1	5.6	14.9	15.0	8.4	15.7	27.2
Indian Head, Saskatchewan	39.8	14.2	27.1	40.2	14.0	23.8	36.1	10.8	27.1	69.0	15.4	51.2
Madison, Wis	16.1	12.3	15.4	15.4	11.8	13.3	8.5	11.3	10.4	22.5	13.7	36.8
Mandan, N. Dak	15.7	17.0	22.8	16.9	17.7	23.6	13.3	14.5	20.0	20.9	16.4	39.8
Moccasin, Mont	13.8	16.1	22.3	14.9	17.6	22.0	14.0	15.2	32.4	17.8	16.1	42.5
Moro, Oreg	10.1	16.3	19.7	8.7	16.3	21.4	11.3	14.5	23.2	10.5	16.4	29.2
North Platte, Nebr	21.3	15.3	17.0	21.1	14.4	16.6	15.8	12.2	17.5	22.5	15.6	28.5
Pendleton, Oreg	27.2	9.3	31.9	32.6	10.0	27.8	35.2	9.6	35.9	27.3	9.5	45.0

BARLEY VARIETIES

	Hannchen selection 1			Trebi			Oderbrucker		
	Acre yield	Protein in grain	Weight of 1,000 kernels	Acre yield	Protein in grain	Weight of 1,000 kernels	Acre yield	Protein in grain	Weight of 1,000 kernels
	Bushels	*Percent*	*Grams*	*Bushels*	*Percent*	*Grams*	*Bushels*	*Percent*	*Grams*
Aberdeen, Idaho	97.0	9.9	39.8	96.4	9.6	43.8	50.5	10.7	35.5
Arlington, Va	12.6	11.0	32.1	11.8	10.5	35.5	4.8	12.7	29.1
Bozeman, Mont	87.2	12.1	38.6	86.3	11.2	43.8	76.6	12.5	36.1
Felt, Idaho	29.4	16.3	31.1	21.5	15.2	34.0	4.8	15.6	29.0
Hays, Kans	11.4	13.2	23.2	47.8	11.7	35.1	9.8	14.4	17.0
Indian Head, Saskatchewan	87.6	14.2	35.3	92.6	12.7	42.7	79.6	13.5	33.7
Madison, Wis	33.0	11.1	32.7	53.2	11.7	40.3	28.9	13.0	29.3
Mandan, N. Dak	29.8	15.3	30.7	19.3	14.6	27.6	16.1	15.1	25.1
Moccasin, Mont	19.6	16.4	25.3	28.2	14.5	29.8	14.8	16.5	25.3
Moro, Oreg	13.1	16.5	27.4	9.8	16.2	22.8	3.5	16.7	22.0
North Platte, Nebr	37.3	12.7	24.2	51.4	12.1	31.8	38.5	12.7	25.5
Pendleton, Oreg	53.5	7.5	39.4	65.1	7.3	42.5	25.6	8.5	34.5

As would be expected, seed produced at the several stations varied greatly in protein content and weight of 1,000 kernels. Kernels of all varieties produced at Aberdeen, Bozeman, Pendleton, and Felt were large, in some cases weighing more than twice as much as those produced elsewhere, as, for example, at Hays, Moccasin, North Platte, and Madison. Seed of most of the varieties from Moccasin, Hays, and Felt was high in protein content, whereas that from Aberdeen, Madison, and Pendleton was low.

In order to obtain seed that differed in size owing to the stage at which it was harvested, a large number of heads in border rows of the Marquis check plots at Moccasin, Mont., were tagged when anthers protruded from florets near the center of the heads. In 1929, the heads were tagged on July 16 and 17 and harvested on July 27 and August 3, 9, and 14, on which dates the kernels were in early

milk, soft-dough, hard-dough, and mature stages, respectively. In 1930, heads were tagged on July 8 and harvested daily from July 12 to 19 and at longer intervals to maturity. The heads were cut from the stems and spread on a screen in the sun to dry rapidly. The weight of 1,000 kernels for each of these samples is given in table 7, in connection with the data on resistance to seedling blight.

The tests for reaction to seedling blight caused by *Gibberella saubinetii* were conducted in soil in the greenhouse at Madison. Considerable difficulty was encountered in perfecting a technique that would give a differential infection. Conidial suspensions used as inoculum proved unsatisfactory. On the other hand, mycelial cultures grown for about 10 days on a medium of 1 part corn meal, 1 part sand, and 1½ parts water, which had been steamed for about 30 minutes and sterilized in an autoclave, produced satisfactory infections. The culture was mixed with sandy soil in the proportion of 150 parts soil to 1 part culture, and 150 cc of the mixture was sown with the seed in each 18-inch row. Strain no. 1[5] of the organism was used in testing the seed lots produced in 1929 and strain no. 73C in testing those produced in 1930. It was not considered necessary to use the same strain each year, as it was desired only to determine whether differences occur in reaction to blight in seedlings of the same variety of wheat or barley from seed produced under different environmental conditions. The tests were conducted in January and February 1930 for the seed lots produced in 1929, and in January and February 1931 for those produced in 1930. Each seed lot tested for reaction to seedling blight was also grown without inoculation to check viability and natural infection of seedling blight organisms. Except as noted otherwise in the table headings, the greenhouses were kept at temperatures averaging about 19° C.

In taking disease notes on the seedlings, the plants were divided into six classes, as follows: (1) Healthy; (2) having lesions on the seminal roots; (3) having lesions on coleoptile, but not stunted; (4) having lesions on coleoptile or deep-seated lesions, and stunted; (5) blighted after emergence; and (6) blighted before emergence (determined by subtracting the number of plants emerging from the number of seeds planted). Cracked kernels were not used, and viability was high in most of the controls. In order to convert the data to a single figure or resistance index for each seed lot, the following values were assigned to each class: Class 1, 1.0; class 2, 0.8; class 3, 0.7; class 4, 0.3; class 5, 0.2; and class 6, 0. These values, on the basis of 100 seeds sown, give a resistance index of 100 for a seed lot that produced 100 healthy plants and 0 for a seed lot that produced no plants.

Tests of wheat for reaction to bunt were conducted in the field at Moccasin and Madison. The seed was shaken in an envelope with an excess of spores from bunted heads of wheat collected at Moccasin. Infection was based on the percentage of heads showing any smut balls.

Yield tests of seed produced at the different locations were conducted in nursery rows at Madison and Moccasin in 1930 and 1931, and at Aberdeen in 1931. The test at Aberdeen was grown under irrigation.

[5] Strain numbers refer to numbers of the collections of *Gibberella saubinetii* of the Department of Plant Pathology, University of Wisconsin.

EXPERIMENTAL RESULTS

REACTION TO SEEDLING BLIGHT

Results With Seed Produced in 1929

All of the seed lots produced at the several stations in 1929 were tested for reaction to seedling blight caused by *Gibberella saubinetii* in two separate tests in the greenhouse at Madison. In each test duplicate rows of 100 seeds each were inoculated; one row was not inoculated in order to determine the viability and natural infection in the seed. In the first test both wheat and barley were sown January 1, 1930, and notes were taken January 20 to 24, when most of the plants were in the third-leaf stage. In the second test the barley was sown February 1 and the wheat February 8, and the notes were taken when most of the plants were in the fourth- or fifth-leaf stage.

The indices of resistance for both tests are given for wheat in table 3 and for barley in table 4. The resistance indices, the weight of 1,000 kernels, and the protein content of the seed sown are shown in figure 1 for the wheat varieties Marquis, Illinois No. 1, Peliss selection 89, and Hard Federation, and in figure 2 for the three barley varieties. In the second test the infection on inoculated wheat was so heavy that resistance indices of seed from several stations approached 0 and differences were obscured; therefore, in figure 1 the data from the first test were used instead of the averages of the two tests.

TABLE 3.—*Reaction to seedling blight, caused by Gibberella saubinetii, of 4 varieties of wheat, from seed produced at 10 experiment stations in 1929, when grown in the greenhouse at Madison, Wis.*

INOCULATED WITH GIBBERELLA SAUBINETII

Station where seed was produced	Resistance index												Average of 4 varieties
	Illinois No. 1			Marquis			Hard Federation			Peliss selection 89			
	First test	Second test	Average	First test	Second test	Average	First test	Second test	Average	First test	Second test	Average	
Madison, Wis.	82.2	43.6	62.9	73.4	32.2	52.8	47.9	28.3	38.1	49.5	[1] 4.9	27.2	45.3
North Platte, Nebr.	62.2	18.2	40.2	57.5	17.8	37.7	55.4	20.8	38.1	60.8	10.8	35.8	38.0
Indian Head, Saskatchewan	70.9	25.0	48.0	49.5	3.9	26.7	33.2	1.1	17.2	42.2	4.6	23.4	28.8
Aberdeen, Idaho	40.1	4.3	22.2	52.6	18.0	35.3	24.4	3.2	13.8	40.3	6.8	23.6	23.7
Bozeman, Mont.	44.3	9.7	27.0	35.5	2.8	19.2	25.7	1.8	13.8	32.0	2.1	17.1	19.3
Moro, Oreg.	43.3	2.5	22.9	42.1	1.5	21.8	27.6	.3	14.0	23.7	.8	12.3	17.8
Hays, Kans.	29.0	.4	14.7	28.7	1.3	15.0	46.4	14.3	30.4	13.6	0	6.8	16.7
Moccasin, Mont.	40.5	2.1	21.3	31.6	.3	16.0	25.5	.6	13.1	25.1	1.4	13.3	15.9
Felt, Idaho	41.7	1.7	21.7	41.0	1.2	21.1	25.7	2.0	13.9	10.6	.3	5.5	15.6
Mandan, N. Dak.	35.1	1.1	18.1	31.9	.3	16.1	23.9	1.9	12.9	23.9	0	12.0	14.8
Average	48.9	10.9	29.9	44.4	7.9	26.2	33.6	7.4	20.5	32.2	3.2	17.7	23.6
CONTROL (NOT INOCULATED)													
Madison, Wis.	97.4	95.5	96.5	95.8	92.4	94.1	92.5	94.1	93.3	86.0	------	------	92.5
North Platte, Nebr.	87.0	97.4	92.2	94.7	94.1	94.4	97.3	97.7	97.5	80.6	87.2	83.9	92.0
Indian Head, Saskatchewan	99.0	97.3	98.2	100.0	98.4	99.2	97.2	98.2	97.7	98.2	98.2	98.2	98.3
Aberdeen, Idaho	91.4	94.3	92.9	97.2	96.5	96.9	92.0	94.5	93.3	90.2	89.9	90.1	93.3
Bozeman, Mont.	89.7	87.1	88.4	93.2	89.7	91.5	81.3	80.8	81.1	92.6	93.7	93.2	88.6
Moro, Oreg.	92.3	92.7	92.5	94.4	88.3	91.4	83.3	85.3	84.3	91.4	80.8	86.1	88.6
Hays, Kans.	92.8	89.9	91.4	95.5	95.4	95.5	89.1	88.8	89.0	76.5	73.5	75.0	87.7
Moccasin, Mont.	92.8	91.7	92.3	91.8	96.4	94.1	92.6	85.6	89.1	83.5	86.6	85.1	90.2
Felt, Idaho	89.8	88.5	89.2	84.6	88.1	86.4	93.1	94.0	93.6	54.8	54.5	54.7	81.0
Mandan, N. Dak.	91.1	91.5	91.3	87.9	81.3	84.6	84.0	95.4	89.7	77.4	82.2	79.8	86.4
Average	92.3	92.6	92.5	93.5	92.1	92.8	90.2	91.4	90.9	83.1	83.0	82.9	89.9

[1] Interpolated.

TABLE 4.—*Reaction to seedling blight, caused by Gibberella saubinetii, of 3 varieties of barley, from seed produced at 10 experiment stations in 1929, when grown in the greenhouse at Madison, Wis.*

Station where seed was produced	Resistance index									
	Hannchen selection 1			Trebi			Oderbrucker			Average of 3 varieties
	First test	Second test	Average	First test	Second test	Average	First test	Second test	Average	
INOCULATED WITH GIBBERELLA SAUBINETII										
Madison, Wis.	76.8	66.6	71.7	81.5	77.8	79.7	84.3	78.7	81.5	77.6
Indian Head, Saskatchewan	70.1	58.2	64.2	67.0	66.0	66.5	67.4	57.2	62.3	64.3
North Platte, Nebr.	67.3	54.7	61.0	64.9	67.0	66.0	66.2	58.3	62.3	63.1
Felt, Idaho	62.0	51.2	56.6	64.5	64.8	64.7	70.0	60.9	65.5	62.3
Bozeman, Mont.	63.1	55.1	59.1	63.0	61.0	62.0	69.0	61.2	65.1	62.1
Aberdeen, Idaho	66.0	57.5	61.8	69.1	56.5	62.8	65.8	56.6	61.2	61.9
Moro, Oreg.	59.1	58.5	58.8	57.0	61.5	59.3	59.8	58.5	59.2	59.1
Hays, Kans.	60.4	56.9	58.7	60.1	56.1	58.1	58.5	56.1	57.3	58.0
Mandan, N. Dak.	61.0	53.6	57.3	62.6	53.1	57.9	57.5	52.5	55.0	56.7
Moccasin, Mont.	55.9	47.5	51.7	57.4	54.2	55.8	57.4	50.8	54.1	53.9
Average	64.2	56.0	60.1	64.7	61.8	63.3	65.6	59.1	62.4	61.9
CONTROL (NOT INOCULATED)										
Madison, Wis.	96.1	87.8	92.0	93.1	93.0	93.1	93.9	92.6	93.3	92.8
Indian Head, Saskatchewan	95.5	95.5	95.5	97.2	97.3	97.3	98.0	98.1	98.1	97.0
North Platte, Nebr.	95.7	91.0	93.4	95.2	95.4	95.3	94.3	94.0	94.2	94.3
Felt, Idaho	91.1	79.5	85.3	94.2	89.3	91.8	97.8	95.8	96.8	91.3
Bozeman, Mont.	94.8	92.1	93.5	96.7	95.0	95.9	95.3	98.9	97.1	95.5
Aberdeen, Idaho	93.2	87.6	90.4	93.9	95.7	94.8	92.8	93.4	93.1	92.8
Moro, Oreg.	94.1	97.4	95.8	90.6	92.8	91.7	96.6	89.8	93.2	93.6
Hays, Kans.	81.8	87.6	84.7	89.8	90.1	90.0	93.9	85.5	89.7	88.1
Mandan, N. Dak.	88.2	91.3	89.8	89.4	85.6	87.5	93.8	91.6	92.7	90.0
Moccasin, Mont.	93.9	91.8	92.9	90.3	91.4	90.9	89.8	94.0	91.9	91.9
Average	92.4	90.2	91.3	93.0	92.6	92.8	94.6	93.4	94.0	92.7

The average resistance index for the four wheat varieties from Madison, as shown in table 3, was 45.3; from North Platte, 38.0; and from Indian Head, 28.8; while those from Mandan, Felt, and Moccasin averaged only 14.8, 15.6, and 15.9, respectively. The average resistance indices of the seed lots from other stations were intermediate. The relative rank of the stations was very similar for the barley varieties. Low viability and natural infection of the seed do not account for the differences, since there were marked differences in reaction in the inoculated series when the rows not inoculated showed similar resistance indices.

The average resistance indices for the seed lots of the four wheat varieties from all stations were as follows: Illinois No. 1, 29.9; Marquis, 26.2; Hard Federation, 20.5; and Peliss selection 89, 17.7. There were no significant differences for the barley varieties.

The differences between seed lots of all varieties produced at different stations were much greater than the average differences between varieties produced at the same stations. Thus for the wheat varieties the greatest difference was between Mandan and Madison, with resistance indices of 14.8 and 45.3, respectively. Among varieties the greatest difference was between Peliss selection 89 and Illinois No. 1, the indices being respectively 17.7 and 29.9. It would appear, therefore, that the place where the seed was grown had more influence on infection than did the variety. This was especially true for the barley, in which the differences between varieties were negligible.

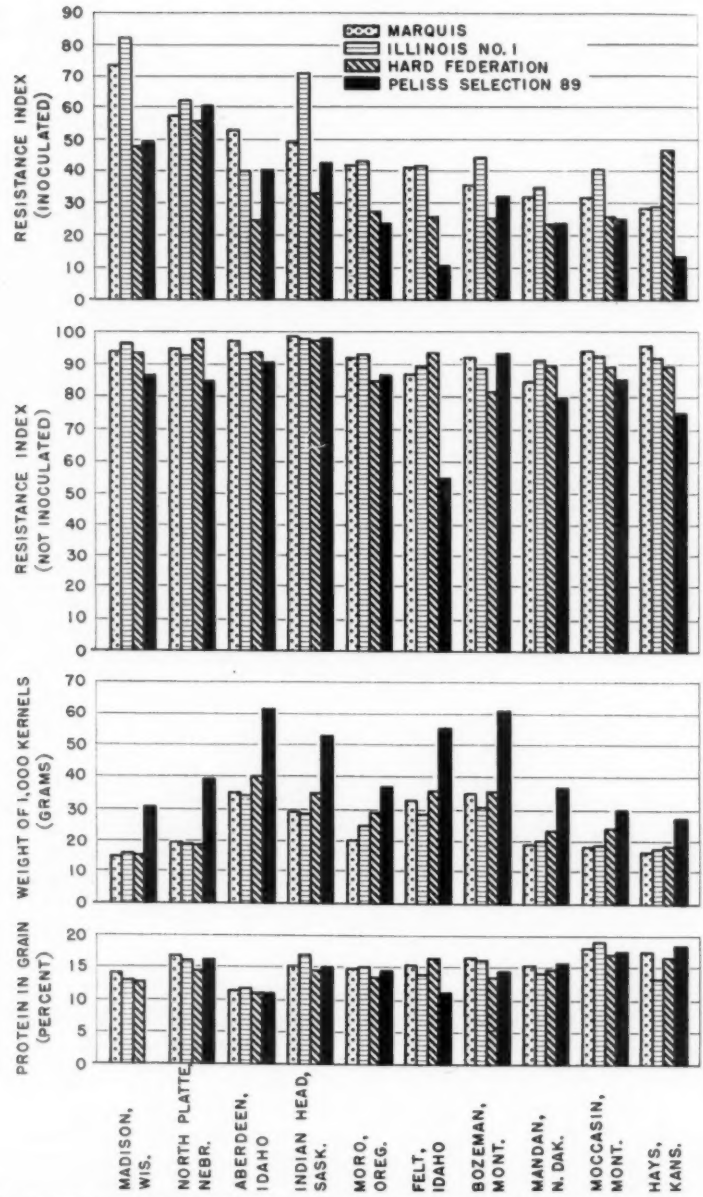

FIGURE 1.—Indices of resistance to seedling blight, caused by *Gibberella saubinetii*, of four varieties of wheat grown in the greenhouse at Madison, Wis., from seed produced at each of 10 experiment stations in 1929, and weight of 1,000 kernels and protein content of seed sown.

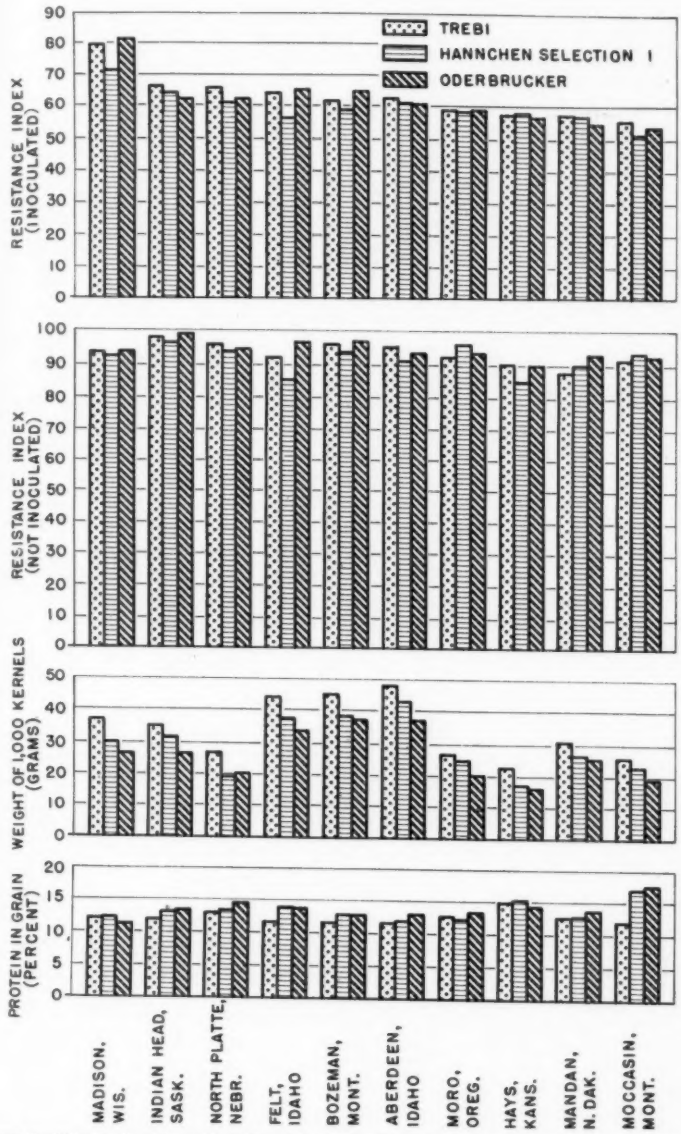

FIGURE 2.—Indices of resistance to seedling blight, caused by *Gibberella saubinetii*, of three varieties of barley grown in the greenhouse at Madison, Wis., from seed produced at each of 10 experiment stations in 1929, and weight of 1,000 kernels and protein content of seed sown.

Standard errors are not given for the data presented in tables 3 and 4, as the ranges in resistance indices are so great that use of a generalized error might be misleading. The significance of the data is indicated, however, by the variability of 11 checks of Marquis distributed throughout the first test. The standard deviation of these checks was 4.21, indicating definitely that the differences in resistance indices mentioned above are not due to random variation.

It will be noted from figure 1 that, excepting the seed lots from Aberdeen and Indian Head, the resistance indices for the inoculated seed of Marquis are only slightly lower than for Illinois No. 1. For seed from Aberdeen the resistance index of Marquis (52.6) was higher than for Illinois No. 1 (40.1), while for seed from Indian Head the resistance index for Marquis (49.5) was much lower than for Illinois No. 1 (70.9). Similar differences are apparent for Peliss selection 89 and Hard Federation. Peliss selection 89 shows a very low resistance index for seed produced at Felt, even when not inoculated. In this case, the grain of Peliss selection 89 was frosted before it was mature and the germination was injured; while the other varieties, being earlier, were ripe when frost occurred and were not injured. Hard Federation from Hays also produced a relatively high resistance index, as compared with the other varieties. This may have been due to its earliness, which allowed it to escape serious injury from heat and drought.

As shown in figure 2, all of the barley varieties were infected less than the wheat varieties and the differences between them were smaller. However, the Madison-grown seed of each variety had the highest resistance index and Moccasin-grown seed the lowest. Seed of all three varieties from the several stations ranked in about the same order in the inoculated seedings, with the exception of Hannchen selection 1 from Felt, which also had a low reading in the uninoculated control. These reactions would suggest, as might be expected, that varieties do not respond alike to all environments.

The weight of 1,000 kernels, the protein content, and the relation of these factors to reaction to seedling blight are discussed in a later section of this paper.

As an additional check on the reliability of the results presented in tables 3 and 4, seed of the 3 barley and 4 wheat varieties from 5 of the 10 experiment stations was grown at temperatures of 12°, 20°, and 26° C. Duplicate rows of 75 seeds each were inoculated with *Gibberella saubinetii* and 1 row of 75 seeds was not inoculated, at each temperature. Results from this test are presented in table 5. The resistance indices of Marquis and Illinois No. 1 grown in the two tests reported in table 3 and an average for those grown under the three temperatures reported in table 5 are shown in figure 3. The results for the inoculated seed lots of these two varieties of wheat from the five stations rank in practically the same order in each test (fig. 3). Results with the other varieties agree equally well. This similarity may be taken as verifying the conclusion that the major differences are not due to random errors.

TABLE 5.—*Reaction to seedling blight, caused by Gibberella saubinetii, of 4 varieties of wheat and 3 varieties of barley, from seed produced at 5 experiment stations in 1929, when grown in the greenhouse at Madison, Wis., at 3 temperatures, in February 1930*

WHEAT VARIETIES

Variety and station where seed was produced	Grown at 26° C.			Grown at 20° C.			Grown at 12° C.			Resistance index average of 3 temperatures	
	Days to emergence	Resistance index		Days to emergence	Resistance index		Days to emergence	Resistance index			
		Inoculated	Not inoculated		Inoculated	Not inoculated		Inoculated	Not inoculated	Inoculated	Not inoculated
Illinois No. 1:											
Madison, Wis.	6	56.0	99.2	6	36.4	93.2	11	46.3	97.1	46.2	96.5
Indian Head, Saskatchewan	5	42.3	97.3	6	37.5	100.0	11	38.8	98.7	39.5	98.7
North Platte, Nebr.	5	28.0	97.9	6	34.6	95.6	10	31.6	91.2	31.4	94.9
Felt, Idaho	6	20.0	94.1	6	15.9	89.8	10	22.3	96.0	19.4	93.3
Moccasin, Mont.	5	19.2	91.5	6	17.0	89.4	10	15.8	89.9	17.3	90.3
Average	5	33.1	96.0	6	28.3	93.6	10	31.0	94.6	30.8	94.7
Marquis:											
Madison, Wis.	9	30.0	96.0	9	25.6	95.5	12	39.0	98.7	31.5	96.7
North Platte, Nebr.	5	32.5	91.9	6	24.9	96.3	11	28.8	94.2	28.7	94.1
Indian Head, Saskatchewan	6	18.7	98.3	6	15.7	97.5	12	18.8	100.0	17.7	98.6
Felt, Idaho	5	16.2	95.2	6	17.0	94.4	11	12.3	95.7	15.2	95.1
Moccasin, Mont.	6	10.6	84.0	6	8.2	83.1	11	8.1	93.3	9.0	86.8
Moccasin, Mont.[1]	7	20.0	97.1	7	8.6	100.0	12	25.4	98.7	18.0	98.6
Average	6	21.3	93.8	7	16.7	94.5	12	22.1	96.8	20.0	95.0
Hard Federation:											
Madison, Wis.	6	14.5	95.5	6	15.3	97.4	12	5.9	96.0	11.9	96.3
North Platte, Nebr.	5	10.2	93.1	6	7.2	92.3	11	8.5	82.9	8.6	89.4
Indian Head, Saskatchewan	5	4.7	98.3	7	3.3	96.4	12	2.2	98.7	3.4	97.8
Moccasin, Mont.	6	3.3	74.7	6	1.2	66.2	12	3.3	80.6	2.6	73.8
Felt, Idaho	6	2.9	89.9	7	2.1	96.2	12	2.5	86.5	2.5	90.9
Average	6	7.1	90.3	6	5.8	89.7	12	4.5	88.9	5.8	89.6
Peliss selection 89:											
North Platte, Nebr.	9	21.1	73.1	9	20.8	73.9	11	20.3	91.3	20.7	79.4
Indian Head, Saskatchewan	11	17.5	88.8	11	10.0	93.2	12	12.8	99.6	13.4	93.9
Felt, Idaho	11	6.1	65.8	12	2.9	58.9	11	4.2	80.5	4.4	68.4
Moccasin, Mont.	12	3.4	72.0	11	4.5	72.9	10	4.7	80.7	4.2	75.2
Average	11	12.0	74.9	11	9.6	74.7	11	10.5	88.0	10.7	79.2

BARLEY VARIETIES

Hannchen selection 1:											
Madison, Wis.	6	47.4	90.7	5	56.4	94.8	8	63.0	94.4	55.6	93.3
Indian Head, Saskatchewan	7	39.7	70.0	5	49.5	69.1	8	51.3	98.0	46.8	79.0
North Platte, Nebr.	5	35.4	72.3	5	46.2	92.5	8	51.8	86.0	44.5	83.6
Felt, Idaho	5	39.3	67.2	5	36.7	88.8	8	46.4	91.5	40.8	82.5
Moccasin, Mont.	8	23.6	80.3	5	39.2	80.5	8	42.0	84.7	34.9	81.8
Average	6	37.1	76.1	5	45.6	85.1	8	50.9	90.9	44.5	84.0
Trebi:											
Madison, Wis.	11	73.4	93.5	6	66.3	88.3	9	77.7	95.9	72.5	92.6
North Platte, Nebr.	7	42.1	95.5	6	43.6	97.5	9	49.6	84.3	45.1	92.4
Indian Head, Saskatchewan	8	35.9	97.1	6	43.1	96.3	9	51.2	98.8	43.4	97.4
Felt, Idaho	7	38.5	92.9	6	41.7	91.2	11	49.1	95.2	43.1	93.1
Moccasin, Mont.	9	30.1	82.7	6	34.8	89.9	9	47.0	88.5	37.3	87.0
Average	8	44.0	92.3	6	45.9	92.6	9	54.9	92.5	48.3	92.5
Oderbrucker:											
Madison, Wis.	5	66.0	95.2	5	71.7	95.6	9	78.3	97.3	72.0	96.0
North Platte, Nebr.	5	50.3	94.1	5	46.3	97.3	9	58.3	95.5	51.6	95.6
Indian Head, Saskatchewan	5	48.9	94.9	5	45.6	97.1	9	47.9	98.7	47.5	96.9
Felt, Idaho	5	33.2	91.6	5	40.6	85.5	9	59.8	92.4	44.5	89.8
Moccasin, Mont.	5	35.5	84.0	5	36.3	90.7	9	48.7	90.8	40.2	88.5
Average	5	46.8	92.0	5	48.1	93.2	9	58.6	94.9	51.2	93.4

[1] Harvested July 27 when grain was in early milk stage.

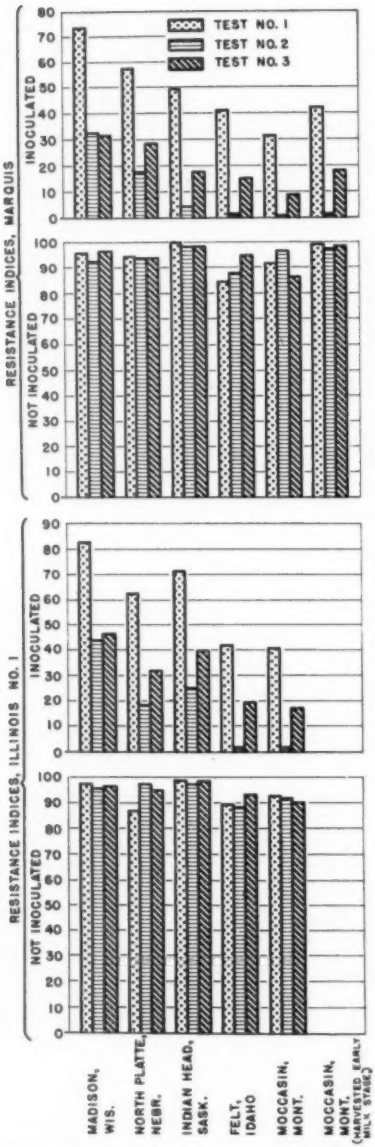

FIGURE 3.—Indices of resistance to seedling blight, caused by *Gibberella saubinetii*, of the wheat varieties Marquis and Illinois No. 1 grown in three tests in the greenhouse at Madison, Wis., from seed produced at each of five experiment stations in 1929.

The average resistance index for all the seed lots for the three temperatures was 30.8 for Illinois No. 1, 20.0 for Marquis, 10.7 for Peliss selection 89, and 5.8 for Hard Federation. The relative reaction of the varieties to seedling blight is the same as in tests 1 and 2 (table 3), except that Peliss selection 89 and Hard Federation are reversed. There was very little difference among the three barley varieties, although Hannchen selection 1 was slightly more susceptible in each test.

An interesting response of varieties to temperature during the period of germination is clearly shown by the results in table 5. When germinated at 12° C., Peliss selection 89 emerged in 11 days and Marquis in 12 days; but when germinated at 26°, Marquis emerged in 6 days, while Peliss selection 89 did not emerge until the eleventh day. Among the three barley varieties, high temperature had a similar retarding effect on Trebi. Apparently this response had no relation to seedling infection in these studies.

Results With Seed Produced in 1930

The resistance indices for the seed lots of the 4 varieties of wheat and 3 varieties of barley produced at 12 experiment stations in 1930 are shown in table 6. Data for the four wheat varieties are shown in figure 4 and for the three barley varieties in figure 5. Four seedings, or a total of 500 kernels, of each seed lot of wheat were grown in inoculated soil; and 2 seedings, one of 75 and one of 100 kernels, were grown without inoculation. Two seedings, or a total of 225 kernels, of each seed lot of barley,

were grown in inoculated soil; while 2 seedings, or a total of 175 kernels, were grown without inoculation.

TABLE 6.—*Reaction to seedling blight, caused by Gibberella saubinetii, of 4 varieties of wheat and 3 varieties of barley from seed produced at 12 experiment stations in 1930, when grown in the greenhouse at Madison, Wis.*

WHEAT VARIETIES

Station where seed was produced	Resistance index									
	Control (not inoculated)					Inoculated with *Gibberella saubinetii*				
	Illinois No. 1A	Marquis	Hard Federation	Peliss selection 89	Average	Illinois No. 1A	Marquis	Hard Federation	Peliss selection 89	Average
Indian Head, Saskatchewan	99.8	97.2	94.2	95.5	96.7	83.2	74.0	79.5	69.3	76.5
Arlington, Va	96.9	94.4	93.4	85.4	92.5	82.6	74.9	74.0	48.9	70.1
Bozeman, Mont	93.0	97.6	95.7	83.3	92.4	76.8	65.6	60.2	62.8	66.4
Madison, Wis	96.0	91.0	81.7	77.4	86.5	83.9	66.1	56.7	44.8	62.9
North Platte, Nebr	90.6	97.4	96.1	92.8	94.2	65.2	68.2	60.9	55.8	62.5
Felt, Idaho	88.2	79.3	71.4	79.6	79.6	74.1	63.7	55.0	55.3	62.0
Mandan, N. Dak	93.4	93.8	88.4	84.5	90.0	75.4	61.0	54.2	42.1	58.2
Aberdeen, Idaho	84.7	93.4	77.1	82.6	84.5	74.2	69.7	37.1	51.3	58.1
Moro, Oreg	83.7	85.2	93.3	91.8	88.5	63.0	57.6	48.6	50.6	55.0
Moccasin, Mont	90.7	83.8	85.4	91.1	87.8	60.9	58.7	38.5	51.2	52.3
Hays, Kans	87.7	90.4	90.8	52.3	80.3	46.9	64.0	59.2	21.0	47.8
Pendleton, Oreg	80.2	85.5	84.0	66.9	79.2	50.4	56.1	33.5	28.9	42.2
Average	90.4	90.8	87.6	81.9	87.7	69.7	65.0	54.8	48.5	59.5

BARLEY VARIETIES

Station where seed was produced	Resistance index							
	Control (not inoculated)				Inoculated with *Gibberella saubinetii*			
	Oderbrucker	Hannchen selection 1	Trebi	Average	Oderbrucker	Hannchen selection 1	Trebi	Average
Indian Head, Saskatchewan	94.3	98.7	96.7	96.6	89.3	80.7	92.7	87.6
Madison, Wis	97.2	88.8	93.4	93.1	93.0	80.7	88.8	87.5
Bozeman, Mont	97.3	94.4	95.0	95.6	86.9	89.2	79.0	85.0
Arlington, Va	97.4	91.0	95.6	94.7	84.0	91.1	78.1	84.4
Felt, Idaho	92.8	95.5	93.1	93.8	83.7	84.0	71.5	79.7
Aberdeen, Idaho	95.2	93.9	93.1	94.1	72.8	77.3	63.0	71.0
Hays, Kans	87.0	90.6	91.9	89.8	67.1	64.3	65.6	65.7
North Platte, Nebr	97.9	92.1	95.4	95.1	71.3	68.3	55.4	65.0
Mandan, N. Dak	99.0	99.1	93.5	97.2	59.2	70.6	64.7	64.8
Moro, Oreg	94.8	94.8	94.1	94.6	66.2	65.5	59.5	63.7
Moccasin, Mont	94.3	92.8	92.8	93.3	57.4	64.2	54.7	58.8
Pendleton, Oreg	94.0	91.3	94.9	93.4	51.4	61.6	52.0	55.0
Average	95.1	93.6	94.1	94.3	73.5	74.8	68.8	72.4

The viability and natural infection of the wheat that was not inoculated were more variable than in tests on the seed produced in 1929; yet after making allowance for this, marked differences in disease reaction of the same variety from seed produced at the different stations are apparent. Seed of most of the varieties of both wheat and barley from Indian Head, Madison, Arlington, and Bozeman showed higher resistance indices than that from Pendleton, Hays, Moccasin, and Moro. The similarity in the response for different

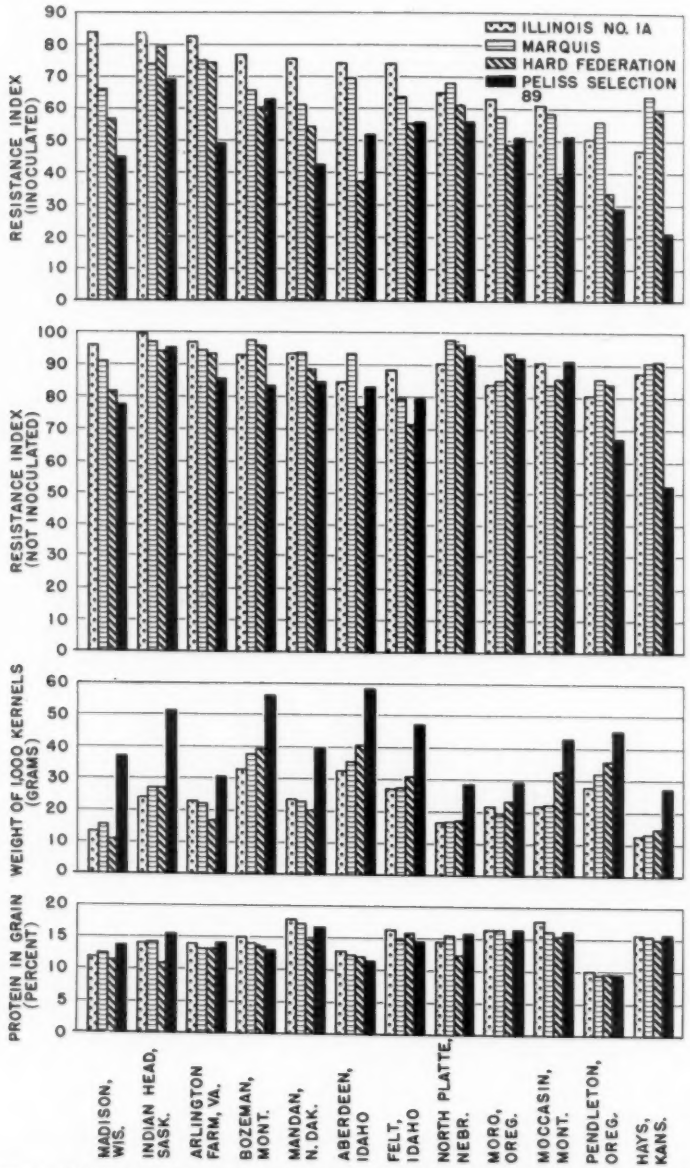

FIGURE 4.—Indices of resistance to seedling blight, caused by *Gibberella saubinetii*, of four varieties of wheat grown in the greenhouse at Madison, Wis., from seed produced at each of 12 experiment stations in 1930, and weight of 1,000 kernels and protein content of seed sown.

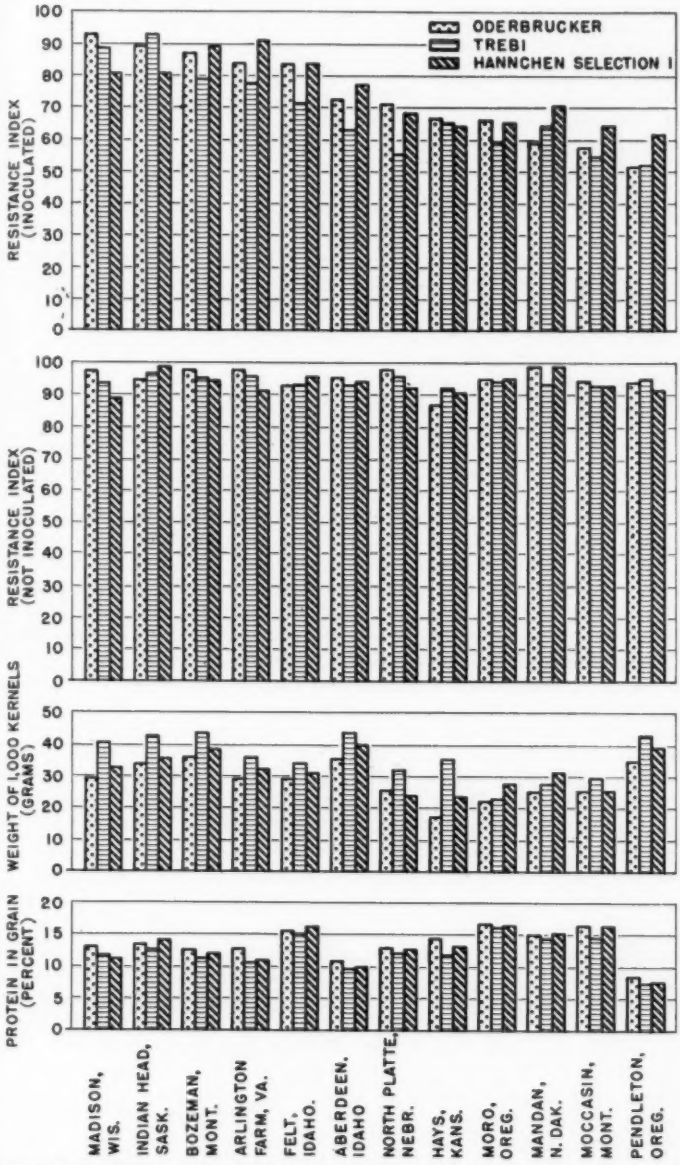

FIGURE 5.—Indices of resistance to seedling blight, caused by *Gibberella saubinetii*, of three varieties of barley grown in the greenhouse at Madison, Wis., from seed produced at each of 12 experiment stations in 1930, and weight of 1,000 kernels and protein content of seed sown.

varieties and the magnitude of the differences are such as to permit little opportunity for explaining the differences on the basis of random errors. For example, the uninoculated rows of Illinois No. 1A had resistance indices of 87.7 and 99.8 from seed produced at Hays and Indian Head, respectively, while in the inoculated series there was a spread from 46.9 to 83.2, or three times that in the control. Trebi produced at Indian Head and Pendleton had resistance indices of 96.7 and 94.9, respectively, when not inoculated; but for the same variety, when inoculated, the resistance indices were 92.7 and 52.0, respectively.

As an average for all stations, the resistance index of inoculated Illinois No. 1A was 69.7; Marquis, 65.0; Hard Federation, 54.8; and Peliss selection 89, 48.5. These resistance indices are relatively the same as for the seed produced in 1929. The differences between resistance indices for the three barley varieties were small, as were those for the 1929 seed.

Differences in the response of the varieties of wheat to environmental conditions are again indicated. For example, seed of Illinois No. 1A (inoculated) produced at Hays and Pendleton had resistance indices of 46.9 and 50.4, as compared with 64.0 and 56.1 for Marquis seed produced at these same stations; whereas, for seed produced at Madison the resistance index of Illinois No. 1 was 83.9 and that of Marquis was 66.1 (table 6 and fig. 4). Hard Federation seed produced at Hays shows a higher resistance index as compared with the other varieties than seed produced at the other stations, as was the case in 1929. Also a relatively lower resistance index was recorded for Hard Federation seed from Aberdeen than from other sources.

Seed lots of the three barley varieties from the several stations rank in about the same order for reaction to seedling blight (fig. 5), except that seed of Hannchen selection 1 from Indian Head and Madison was relatively more susceptible than the other two varieties. Seed of the varieties of wheat and barley produced at the several stations also ranked in about the same order at each station for weight of 1,000 kernels and protein content, although there are some cases in which the varieties show a differential response to environment. A notable example of this is the relatively large kernel weight of Trebi produced at Hays.

Comparison of Seed Produced in 1929 and 1930

Since it has been shown that varieties in some cases differ markedly in response to environment at the different stations and that averages of the varieties tend to obscure these differences, it seems best in comparing the seedling blight reaction of seed produced at the several stations in 1929 and 1930 to compare the varieties separately rather than to compare averages. Also, as mentioned earlier, the results from test no. 1 show the true range in disease reaction to inoculation more accurately than those from test no. 2, in the case of the wheat varieties produced in 1929; therefore only data from test no. 1 were used in this comparison. The average data from the two tests of barley were used.

The resistance indices of the seed of Illinois No. 1 and 1A wheat and Trebi barley produced at the several experiment stations in 1929

and 1930 are shown in figure 6. For the wheat the highest resistance index each year was that of seed produced at Madison and the lowest was that of seed produced at Hays; but resistance indices for seed from some stations, notably that from Bozeman, Aberdeen, Felt, and Mandan were relatively higher in 1930 than in 1929. The seed of Trebi barley from Indian Head and from Bozeman had a relatively higher resistance index in 1930 than in 1929, while that from North Platte had a relatively lower index. Similar results were obtained with the other varieties. For each of the varieties the protein content and especially the weight of 1,000 kernels produced at the several stations were similar for the 2 years. Apparently neither of these factors will account for the relative differences in reaction to seedling blight between the samples from the several stations for 2 years.

Although samples were obtained for only 2 years, it would seem that seed from some locations may be on the average more resistant to seedling blight over a period of years than seed from other locations but that in any one year the relative disease reaction may be reversed.

Effect of Premature Harvest

The number of days from flowering, percentage of protein in the seed, weight of 1,000 kernels, and indices of resistance to seedling blight for the seed lots of Marquis wheat harvested at several stages of maturity at Moccasin in 1929 and 1930, are presented in table 7. The resistance indices for seed produced in 1929 are from duplicate inoculated rows and from one uninoculated row in each of two seedings in the greenhouse at Madison in January and February 1930. One hundred kernels were sown in each row. For the seed produced in 1930 there were 4 seedings, or a total of 500 kernels, of each lot grown in inoculated soil; and 2 seedings, one of 75 and one of 100 kernels, grown without inoculation. Tests on 1930 seed were conducted in the greenhouse at Madison, in January and February 1931.

Table 7.—*Weight of 1,000 kernels, percentage of protein, and indices of resistance to seedling blight of Marquis wheat harvested at several stages of maturity at Moccasin, Mont., in 1929 and 1930*

Date harvested [1]	Period from flowering to harvest	Protein	Weight of 1,000 kernels	Resistance index after indicated treatment of seed		Date harvested [1]	Period from flowering to harvest	Protein	Weight of 1,000 kernels	Resistance index after indicated treatment of seed	
				Not inoculated	Inoculated					Not inoculated	Inoculated
1929	*Days*	*Percent*	*Grams*			*1930*	*Days*	*Percent*	*Grams*		
July 27	11	15.2	9.0	98.6	21.5	July 15	7	.....	5.5	94.4	67.7
Aug. 3	18	17.3	15.0	99.0	24.5	July 16	8	.....	6.8	96.6	80.0
Aug. 9	24	17.9	16.3	92.1	22.5	July 17	9	.....	8.0	97.3	81.8
Aug. 14	29	18.4	16.8	96.2	20.7	July 18	10	.....	9.1	98.6	85.1
1930						July 19	11	.....	10.6	98.6	80.1
July 12	4	.....	2.4	68.8	23.0	July 23	15	.....	15.9	97.6	84.9
July 13	5	.....	3.1	90.8	49.2	July 28	20	.....	20.4	99.3	90.1
July 14	6	.....	4.3	90.4	64.9	Aug. 1	24	.....	21.5	97.6	90.3
						Aug. 10	33	.....	22.1	99.4	83.0

[1] Heads were tagged on July 16 and 17, 1929, and July 8, 1930, when anthers protruded from florets near the middle of the heads, and were harvested on the dates indicated.

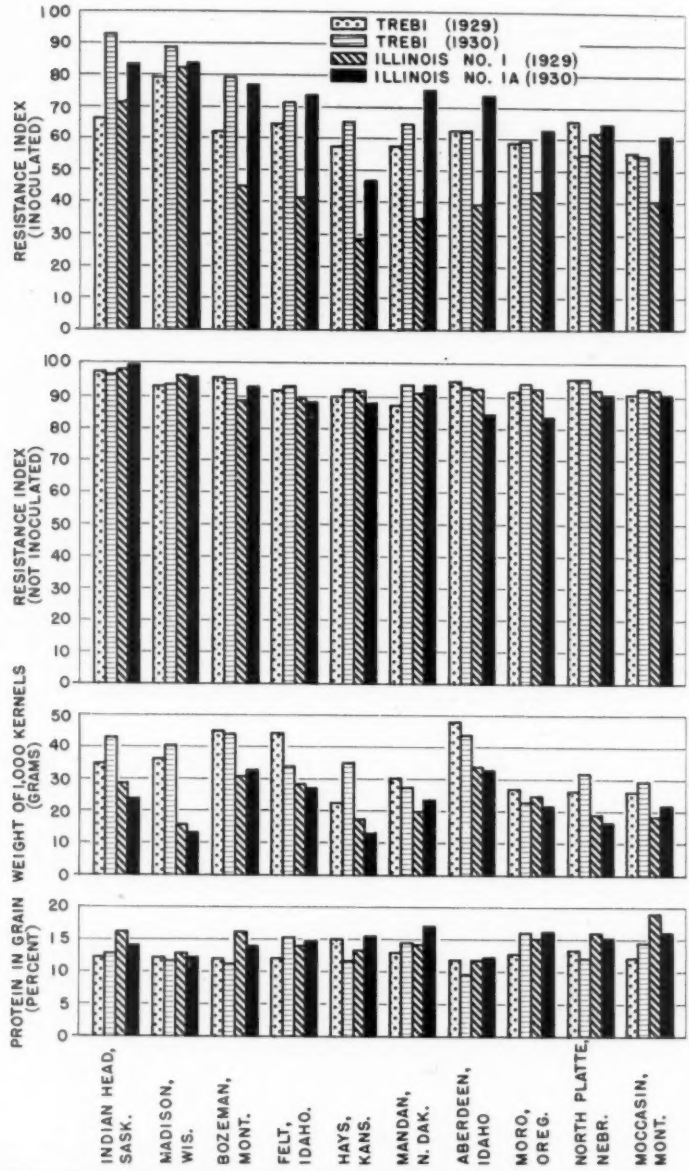

FIGURE 6.—Indices of resistance to seedling blight, caused by *Gibberella saubinetii*, of Trebi barley and Illinois No. 1 or Illinois No. 1A wheat, grown in the greenhouse at Madison, Wis., from seed produced at each of 10 experiment stations in 1929 and 1930, and weight of 1,000 kernels and protein content of seed sown.

Although the weight of 1,000 kernels of the 1929 crop ranged from 9.0 to 16.8 g, depending on the date of harvest, there were no significant differences in disease reaction of inoculated seed. Likewise, seed harvested on July 18, 1930, 10 days after flowering, which weighed only 9.1 g per 1,000 kernels, had a resistance index, when inoculated, equal to that of mature seed, which weighed 22.1 g per 1,000 kernels. On the other hand, seed harvested from the fourth to the seventh day after flowering was much more susceptible. This seed ranged in weight from 2.4 to 5.5 g per 1,000 kernels, and for this group the resistance index increased with the weight of the seed. The fact that kernels harvested only 11 days after blooming in 1929 and 10 days after blooming in 1930 had resistance indices equal to those of mature seed suggests that the differences in disease reaction noted for seed produced at different stations were not due to degree of maturation of the kernels. This also is indicated by the fact that seed badly shriveled by premature ripening at some stations (Madison and North Platte) was more resistant than plump, well-developed seed of the same variety from other stations (Aberdeen and Pendleton).

Effect of Seed Size and Protein Content

There is some suggestion in the data presented (figs. 1, 2, 4, 5) that reaction to seedling blight might be dependent upon or related to the protein content and size of the seed sown. In order to study this relation more critically, the correlation coefficients for indices of resistance and these two characters were calculated. The correlation coefficients for protein content and size of seed were also calculated in order to determine the partial correlation coefficients. It was observed, as may be noted in figures 4 and 5, that the seed lots from Pendleton were the lowest in protein content and among the highest in weight of 1,000 kernels; yet they showed the lowest resistance indices for each of the barley varieties and for 3 of the 4 wheat varieties. Since this relation was different, it appeared that the seed differed from that from other stations in some important but unknown respect, and correlation coefficients for 1930 were also calculated with the omission of Pendleton results. Correlation coefficients were first determined for each variety separately. Since they did not differ significantly for the barley varieties or for the wheat varieties except Hard Federation, a single correlation coefficient was calculated for the wheat varieties Marquis, Illinois No. 1, and Peliss selection 89, and for the barley varieties Trebi, Hannchen selection 1, and Oderbrucker. The correlation for the combined varieties was calculated from the z values of the correlations for the single varieties in order to correct for the means, which differed for the varieties. The correlation coefficients for the three varieties combined and those for each variety individually, as well as the means for the characters correlated, are given in table 8 for the seed lots produced in 1929 and in 1930. Partial correlation coefficients are given in table 9.

TABLE 8.—*Coefficients of correlation between indices of resistance to seedling blight, percentage of protein, and weight of 1,000 kernels seeded, and means for the 3 characters for varieties of wheat and barley from seed produced at several experiment stations in 1929 and 1930*

Year seed was produced and variety	Seed lots	Means			Resistance with protein		Resistance with kernel weight		Protein with kernel weight	
		Resistance indices	Protein in grain	Weight of 1,000 kernels	Correlation coefficient	*P* value	Correlation coefficient	*P* value	Correlation coefficient	*P* value
1929	*Number*		*Percent*	*Grams*						
Hard Federation	10	33.6	14.5	27.4	+0.0486	0.89	−0.7222	0.02	−0.3018	0.40
Marquis	14	43.8	16.0	21.2	−.4992	.07	−.0148	.96	−.3873	.18
Illinois No. 1	10	48.9	15.0	23.7	+.0824	.82	−.1661	.65	−.1273	.73
Peliss selection 89	9	30.2	14.9	44.3	−.0100	.98	+.2331	.55	−.8174	<.01
3 wheat varieties	33				−.2257	.26	+.0037	.99	−.4713	<.01
Hannchen selection 1	10	60.1	13.7	29.0	−.6674	.04	+.1652	.65	−.4684	.18
Oderbrucker	10	62.4	14.0	26.2	−.7323	.02	+.3175	.37	−.4378	.21
Trebi	10	63.3	12.7	34.1	−.2571	.47	+.3242	.36	−.7171	.02
3 barley varieties	30				−.5834	<.01	+.2704	.20	−.5555	<.01
1930 [1]										
Hard Federation	12	54.8	13.1	25.7	−.0339	.92	−.4930	.10	−.0659	.84
Marquis	12	65.0	14.2	24.3	−.1089	.74	+.0386	.91	−.4217	.18
Illinois No. 1A	12	69.7	14.6	23.1	−.0113	.93	+.2108	.51	−.0751	.82
Peliss selection 89	12	48.5	14.3	41.0	+.1896	.56	+.4547	.14	−.5057	.09
3 wheat varieties	36				+.0238	.90	+.2427	.20	−.3458	.07
Hannchen selection 1	12	74.8	13.0	31.7	−.1130	.73	+.4416	.16	−.6288	.03
Oderbrucker	12	73.6	13.5	28.5	+.0158	.96	+.3448	.28	−.6450	.03
Trebi	12	68.8	12.3	35.8	+.0008	.99	+.4522	.15	−.7852	<.01
3 barley varieties	36				−.0323	.87	+.4139	.02	−.6937	<.01
1930 [2]										
Hard Federation	11	56.7	13.4	24.8	−.4241	.20	−.4081	.22	+.1584	.64
Marquis	11	65.8	14.6	23.6	−.6425	.04	+.2165	.52	−.2983	.37
Illinois No. 1A	11	71.5	15.0	22.7	−.4641	.16	+.3806	.25	+.0880	.08
Peliss selection 89	11	50.3	14.7	40.6	−.2321	.49	+.5770	.07	−.6370	.04
3 wheat varieties	33				−.4625	.02	+.4021	.04	−.3133	.12
Hannchen selection 1	11	76.0	13.5	31.0	−.4499	.17	+.7372	<.01	−.4722	.15
Oderbrucker	11	75.5	14.0	28.0	−.4855	.14	+.6203	.04	−.6017	.05
Trebi	11	70.3	12.7	35.2	−.3269	.33	+.6533	.03	−.7859	<.01
3 barley varieties	33				−.4230	.03	+.6735	<.01	−.6389	<.01

[1] Data from all stations included.
[2] Data from Pendleton, Oreg., omitted.

TABLE 9.—*Partial correlation coefficients between indices of resistance to seedling blight and protein content and weight of 1,000 kernels seeded, for seed lots of wheat and barley produced at several experiment stations in 1929 and 1930*

Year seed was produced and variety	Resistance with protein (kernel weight held constant)		Resistance with kernel weight (protein content held constant)	
	Partial correlation coefficient	*P*	Partial correlation coefficient	*P*
1929				
3 wheat varieties	−0.2540	0.21	−0.1195	0.56
Hard Federation wheat	−.2567	.51	−.7430	.02
3 barley varieties	−.5411	<.01	−.0795	.72
1930 [1]				
3 wheat varieties	−.3870	.05	+.3055	.14
Hard Federation wheat	−.3988	.26	−.3813	.28
3 barley varieties	+.0128	.95	+.5784	<.01
1930 [2]				
3 wheat varieties	+.1183	.54	+.2675	.17
Hard Federation wheat	−.0765	.82	−.4965	.13
3 barley varieties	+.3886	.04	+.4839	.01

[1] Data from Pendleton, Oreg., omitted. [2] Data from all stations included.

The correlation coefficients for resistance indices and protein content for wheat produced in 1929 are not significant for any variety taken separately nor for the three varieties considered together. The negative correlation (-0.4992, $P=0.07$) for Marquis approaches significance. Substantially the same is true for seed produced in 1930 when all stations are included. However, when Pendleton is omitted the correlation coefficients for all the varieties individually are negative and that for Marquis (-0.6425, $P=0.04$) and for the three wheat varieties combined (-0.4625, $P=0.02$) are significant.

Further light on the relationship between reaction to seedling blight and protein content is shown by the partial correlation coefficients given in table 9. When size of seed is held constant, Hard Federation shows a negative, though not statistically significant, partial correlation coefficient for protein content and resistance indices for seed produced in 1929 (-0.2567, $P=0.51$) and in 1930 (-0.3988, $P=0.26$), when Pendleton is omitted. The other three wheat varieties also show a small negative partial correlation coefficient (-0.2540, $P=0.21$) for seed lots produced in 1929 and a statistically significant negative partial correlation coefficient (-0.3870, $P=0.05$) for seed lots produced in 1930, when Pendleton is omitted.

The correlation coefficient for resistance indices and protein content for two of the three barley varieties for seed produced in 1929 is negative and significant (table 8). The coefficient for the third variety is also negative though not significant. For all three varieties taken together the coefficient is -0.5834, $P<0.01$. For the 1930 seed, when results from Pendleton are omitted all of the correlation coefficients for the varieties taken individually are negative, although none are significant. For all three varieties taken together the coefficient is -0.4230, $P=0.03$. Holding size of seed constant (table 9), a significant negative partial correlation coefficient (-0.5411, $P<0.01$) is obtained for the three barley varieties produced in 1929, while for the 1930 seed, with Pendleton omitted, the partial correlation coefficient is very small and not significant. It would appear from these data that a negative relation exists between protein content of the seed sown and resistance to seedling blight caused by *Gibberella saubinetii*, but that its influence may be overbalanced by other factors.

Since, as will be shown later, a high correlation ($+0.9312$, $P<0.01$ for wheat, and $+0.8213$, $P<0.01$ for barley) exists between size of seed and dry weight of seedlings, a high correlation might be expected between resistance to seedling blight and size of seed. In general, the data support this assumption but they are not entirely consistent. Thus the correlation coefficients for individual varieties are positive for 10 of the 14 variety years but are statistically significant only for barley varieties produced in 1930. No statistically significant correlations were found for the wheat varieties Marquis, Illinois No. 1, and Peliss selection 89 for seed lots produced in 1929. The correlation coefficient for seed of these varieties produced in 1930 is $+0.2427$, $P=0.20$, for all seed lots and $+0.4021$, $P=0.04$, when those from Pendleton are omitted. Hard Federation, however, shows a significant negative correlation coefficient (-0.7222, $P=0.02$) for seed produced in 1929 and a negative, though not statistically significant, correlation coefficient (-0.4081, $P=0.22$) for 1930 seed when Pendleton is omitted. Holding protein content constant, the partial correlation coefficients for Marquis, Illinois No. 1A, and Peliss selection

89 show little or no relation between size of seed and resistance indices for seed produced in either year. A significant negative partial correlation coefficient (-0.7430, $P=0.02$) was found for Hard Federation seed produced in 1929 and a negative but not statistically significant partial correlation coefficient (-0.3813, $P=0.28$) for 1930 seed. It does not seem reasonable that small seed as such is correlated with resistance indices in the Hard Federation variety but rather that some other condition closely associated with size of seed is concerned. The nature of this condition is not apparent from the present studies.

The three barley varieties show a positive but not statistically significant correlation coefficient ($+0.2704$, $P=0.20$) between size of seed and resistance to seedling blight for seed lots produced in 1929, and a significant positive correlation coefficient ($+0.4139$, $P=0.02$) for all seed lots produced in 1930. When data on the seed lots from Pendleton are omitted the correlation coefficient becomes $+0.6735$, $P<0.01$. When protein content is held constant, partial correlation coefficients show that size of seed in barley was not correlated with resistance indices in 1929 seed lots, but was rather high for 1930 seed lots when all stations were included ($+0.4839$, $P=0.01$) and when Pendleton was omitted ($+0.5784$, $P<0.01$). These data indicate that size of seed as well as protein content may sometimes influence the reaction of wheat and barley to seedling blight.

REACTION TO BUNT

Seed of Marquis, Hard Federation, Illinois No. 1, and Illinois No. 1A, produced at each of the several experiment stations, was inoculated with bunt spores and grown in duplicate 8-foot rows at Moccasin in 1930 and 1931, and at Madison in 1931. Results from these tests are given in table 10. A generalized standard error was calculated for each variety tested at each station, and the standard error of a difference for the averages was computed from these.

TABLE 10.—*Percentage of bunt in three varieties of spring wheat grown at Moccasin, Mont., and Madison, Wis., from seed produced at several experiment stations in 1929 and 1930*

Variety, year, and station on which seed was produced	Percentage of bunt at—				
	Moccasin, Mont.		Madison, Wis., 1931	Average, 1931	Average of 3 station years
	1930	1931			
Hard Federation:					
1929:					
Indian Head, Saskatchewan	93	83	79	81.0	85.0
Hays, Kans.	86	84	81	82.5	83.7
North Platte, Nebr.	87	89	70	79.5	82.0
Mandan, N. Dak.	90	77	77	77.0	81.3
Moro, Oreg.	78	72	76	74.0	75.3
Moccasin, Mont.	76	91	55	73.0	74.0
Bozeman, Mont.	77	81	59	70.0	72.3
Felt, Idaho	76	77	57	67.0	70.0
Aberdeen, Idaho	65	82	60	71.0	69.0
1930:					
North Platte, Nebr.	----	82	82	82.0	----
Mandan, N. Dak.	----	85	78	81.5	----
Arlington, Va.	----	81	79	80.0	----
Bozeman, Mont.	----	87	67	77.0	----
Hays, Kans.	----	84	67	75.5	----
Felt, Idaho	----	75	73	74.0	----
Madison, Wis.	----	76	70	73.0	----
Moccasin, Mont.	----	74	69	71.5	----
Moro, Oreg.	----	81	62	71.5	----
Indian Head, Saskatchewan	----	80	61	70.5	----
Aberdeen, Idaho	----	77	62	69.5	----
Pendleton, Oreg.	----	79	58	68.5	----
Standard error of a difference	----	----	----	10.2	9.6

TABLE 10.—*Percentage of bunt in three varieties of spring wheat grown at Moccasin, Mont., and Madison, Wis., from seed produced at several experiment stations in 1929 and 1930*—Continued

Variety, year, and station on which seed was produced	Percentage of bunt at—				
	Moccasin, Mont.		Madison, Wis., 1931	Average, 1931	Average of 3 station years
	1930	1931			
Illinois No. 1:					
1929:					
Hays, Kans	67				
Madison, Wis	50	90	22	56.0	54.0
North Platte, Nebr	52	75	35	55.0	54.0
Moccasin, Mont	50	70	37	53.5	52.3
Bozeman, Mont	52	69	17	43.0	46.0
Indian Head, Saskatchewan	46	65	27	46.0	46.0
Mandan, N. Dak	49	66	21	43.5	45.3
Felt, Idaho	39	68	26	47.0	44.4
Aberdeen, Idaho	41	66	23	44.5	43.3
Moro, Oreg	39	54	24	39.0	39.0
Standard error of a difference				9.3	8.3
Illinois No. 1A:					
1930:					
North Platte, Nebr		81	75	78.0	
Indian Head, Saskatchewan		86	62	74.0	
Felt, Idaho		86	49	67.5	
Bozeman, Mont		82	49	65.5	
Hays, Kans		82	49	65.5	
Arlington, Va		74	52	63.0	
Mandan, N. Dak		84	40	62.0	
Aberdeen, Idaho		77	44	60.5	
Moro, Oreg		66	48	57.0	
Moccasin, Mont		77	33	55.0	
Pendleton, Oreg		60	44	52.0	
Madison, Wis		66	24	45.0	
Standard error of a difference				9.3	
Marquis:					
1929:					
Moccasin, Mont. (July 27) [1]	37	54	11	32.5	34.0
Mandan, N. Dak	24	54	17	35.5	31.7
Moccasin, Mont. (Aug. 14) [1]	27	48	15	31.5	30.0
Madison, Wis	24	52	13	32.5	29.7
North Platte, Nebr	31	49	7	28.0	29.0
Moro, Oreg	22	55	9	32.0	28.7
Moccasin, Mont. (Aug. 3) [1]	30	48	7	27.5	28.3
Moccasin, Mont. (Aug. 9) [1]	26	45	12	28.5	27.7
Hays, Kans	30	46	3	24.5	26.3
Bozeman, Mont	16	48	6	27.0	23.3
Aberdeen, Idaho	19	44	4	24.0	22.3
Felt, Idaho	14	41	5	23.0	20.0
Indian Head, Saskatchewan	13	36	8	22.0	19.0
1930:					
Moro, Oreg		52	13	32.5	
North Platte, Nebr		52	13	32.5	
Madison, Wis		58	4	31.0	
Mandan, N. Dak		47	10	28.5	
Indian Head, Saskatchewan		46	6	26.0	
Arlington, Va		42	8	25.0	
Felt, Idaho		43	3	23.0	
Hays, Kans		42	4	23.0	
Bozeman, Mont		38	4	21.0	
Aberdeen, Idaho		38	2	20.0	
Pendleton, Oreg		35	4	19.5	
Moccasin, Mont.:					
Aug. 10 [1]		36	4	20.0	
Aug. 1 [1]		38	5	21.5	
July 28 [1]		28			
July 23 [1]		29	4	16.5	
July 19 [1]		38	2	20.0	
July 18 [1]		52	2	27.0	
July 17 [1]		28	6	17.0	
July 16 [1]		45	2	23.5	
July 15 [1]		51	4	27.5	
July 14 [1]		45	3	24.0	
Standard error of a difference				5.9	6.1

[1] Heads were tagged at Moccasin, Mont., on July 16, 1929, and July 8, 1930, when anthers were protruded from florets near the center of the heads, and were harvested on the dates indicated.

No significant differences in bunt infection were observed for Illinois No. 1 produced at the several experiment stations in 1929 nor for Hard Federation produced in either year. A difference of 33.0 percent in infection was recorded for the seed lots of Illinois No. 1A produced at North Platte (78.0 percent), and at Madison (45.0 percent), in 1930. This difference (3.5 times its standard error) was the only one for this variety that was more than twice the standard error. As an average for the three tests, the Marquis wheat produced at Felt and at Indian Head showed less bunt infection than seed harvested July 27 at the early milk stage at Moccasin, the differences being 2.3 and 2.5 times the standard error respectively. Considering the data for the seed lots of Marquis produced at the several experiment stations, when grown in the tests at Moccasin and Madison in 1931, the greatest difference is between that produced at Mandan in 1929 and that produced at Pendleton in 1930. The average difference in infection is 16.0 percent, which is 2.7 times the standard error. There are no statistically significant differences between seed lots of Marquis harvested at several stages of maturity at Moccasin, although there is a tendency for the seed lots with smaller kernels to be more susceptible.

A check row of Marquis from seed produced at Aberdeen in 1930 was sown after each nine seed lots in the bunt tests conducted in 1931. The greatest difference in bunt infection between any two checks was 12, which is 2.6 times the standard error. Considering the number of comparisons and the variability in the checks, it seems doubtful if any of the differences are really significant, except possibly the difference between the two seed lots mentioned of Illinois No. 1A.

A further test was made by calculating correlation coefficients for weight of 1,000 kernels sown and the average percentage of bunt in the tests at Moccasin and Madison in 1931. The correlation coefficients were -0.5358, $P=0.01$, for 21 lots of Hard Federation; -0.1861, $P=0.42$, for 21 lots of Illinois No. 1 and No. 1A combined; and -0.2143, $P=0.23$, for 33 lots of Marquis. When the coefficients for the three varieties were combined, a small but statistically significant coefficient (-0.3036, $P=0.01$) was obtained. These coefficients would seem to indicate that size of seed sown does affect the reaction of a given variety to bunt. That it is not the only factor nor a very important one is evidenced by the small and in most cases nonsignificant correlation coefficient and by the fact that seed of Illinois No. 1A produced at Madison had the lowest bunt infection of any seed lot of this variety and yet had the smallest kernels.

VIGOR OF SEEDLINGS AND YIELD OF GRAIN

Yield tests were conducted in 1930 and 1931 at Moccasin, where each seed lot was grown in four nursery plots; in 1930 and 1931 at Madison; and in 1931 at Aberdeen. At Madison and Aberdeen each seed lot was grown in three nursery plots. Approximately 400 kernels were sown in each 18-foot row, so that stands were about equal for each seed lot. Seeding was done at the normal seeding time at each station. At each station 16 feet from the center row of each three-row plot was harvested for yield. The dry weight of 100 seedlings at about the five-leaf stage from each seed lot was also determined from healthy plants in the uninoculated rows grown with the seedling

blight tests in the greenhouse at Madison. The weight of 1,000 kernels of the seed sown, the dry weight of the seedlings, and the yield in 1930 of the seed lots produced in 1929 are given in table 11. In addition to the above-mentioned tests, seed of three varieties of wheat and one of barley produced at each of four stations in 1929 was again tested for yield in 1931 at Madison, Moccasin, and Aberdeen, together with seed of the same varieties produced at the same stations in 1930. The results are presented in table 12.

TABLE 11.—*Yields at Madison, Wis., and Moccasin, Mont., in 1930, of the seed lots of 4 varieties of wheat and 3 of barley, produced at 10 experiment stations in 1929, weight of 1,000 kernels of the seed sown, and dry weight of 100 seedlings at fifth-leaf stage in the greenhouse at Madison, Wis.*

WHEAT VARIETIES

Variety and station where seed was produced	Weight of 1,000 kernels sown	Dry weight of 100 seedlings	Yield of grain per 16-foot row		
			Moccasin, Mont.	Madison, Wis.	Average
Marquis:	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Moccasin, Mont.[1 2]	18.0	2.78	139	179	159
Do.[1 3]	15.0	2.37	162	156	159
Felt, Idaho	32.8	4.06	121	196	159
Indian Head, Saskatchewan	29.0	3.92	136	178	157
Mandan, N. Dak	19.2	2.72	138	172	155
Aberdeen, Idaho	35.1	4.10	129	179	154
Moccasin, Mont.[1 4]	16.3	2.65	163	138	151
Bozeman, Mont	34.8	4.39	129	168	149
Moro, Oreg	20.2	2.76	132	157	145
Hays, Kans	16.6	2.29	128	151	140
Moccasin, Mont.[1 5]	9.0	1.48	151	120	136
Madison, Wis	14.8	2.01	125	144	135
North Platte, Nebr	19.4	2.92	123	145	134
Illinois No. 1:					
Madison, Wis	15.8	2.30	147	161	154
Bozeman, Mont	30.5	4.47	156	142	149
Felt, Idaho	28.6	4.24	132	158	145
Aberdeen, Idaho	34.0	4.48	140	145	143
Hays, Kans	17.4	2.72	107	153	130
Indian Head, Saskatchewan	28.5	4.22	136	121	129
Moccasin, Mont	18.4	3.16	137	121	129
Mandan, N. Dak	20.0	3.02	137	105	121
North Platte, Nebr	19.0	3.22	126	109	118
Moro, Oreg	24.8	2.72	129	93	111
Feliss selection 89:					
Moccasin, Mont	29.7	3.17	176	309	243
Aberdeen, Idaho	61.1	4.55	180	269	225
Bozeman, Mont	60.6	4.85	169	264	217
Moro, Oreg	37.0	3.04	176	247	212
Felt, Idaho	55.2		162	253	208
Indian Head, Saskatchewan	52.4	4.38	179	225	202
North Platte, Nebr	39.1	3.63	171	214	193
Mandan, N. Dak	36.3	3.23	173	213	193
Hays, Kans	27.0	2.58	180	205	193
Hard Federation:					
Aberdeen, Idaho	39.8	3.21	147	104	126
Indian Head, Saskatchewan	34.8	3.59	151	100	126
Hays, Kans	18.2	2.88	142	103	123
Bozeman, Mont	35.6	3.72	137	99	118
Mandan, N. Dak	23.0	3.22	134	97	116
Felt, Idaho	35.6	3.81	127	96	112
Moro, Oreg	28.9	3.29	134	88	111
North Platte, Nebr	18.8	2.25	132	86	109
Moccasin, Mont	24.2	2.95	129	85	107
Madison, Wis	15.2	1.93	92	70	81
Standard error of a difference			19.3	28.2	24.5

[1] Heads were tagged on July 8, when anthers were protruded from florets near the center of the heads, and harvested at the indicated stage of development.
[2] Mature stage.
[3] Soft-dough stage.
[4] Hard-dough stage.
[5] Early milk stage.

TABLE 11.—*Yields at Madison, Wis., and Moccasin, Mont., in 1930, of the seed lots of 4 varieties of wheat and 3 of barley, produced at 10 experiment stations in 1929, weight of 1,000 kernels of the seed sown, and dry weight of 100 seedlings at fifth-leaf stage in the greenhouse at Madison, Wis.*—Continued

BARLEY VARIETIES

Variety and station where seed was produced	Weight of 1,000 kernels sown	Dry weight of 100 seedlings	Yield of grain per 16-foot row		
			Moccasin, Mont.	Madison, Wis.	Average
Hannchen selection 1:	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Felt, Idaho	37.7	4.71	172	355	264
Hays, Kans	17.2	2.45	173	322	248
Indian Head, Saskatchewan	31.2	3.74	150	338	244
Moccasin, Mont	23.2	3.14	163	315	239
North Platte, Nebr	19.2	2.90	148	329	239
Madison, Wis	29.4	3.25	152	321	237
Bozeman, Mont	38.3	3.53	196	275	236
Mandan, N. Dak	26.3	2.78	185	284	235
Aberdeen, Idaho	42.8	3.93	170	297	234
Moro, Oreg	24.9	2.67	173	277	225
Oderbrucker:					
Bozeman, Mont	37.2	4.97	153	395	274
Aberdeen, Idaho	37.2	4.80	115	376	246
North Platte, Nebr	20.3	3.09	132	323	228
Hays, Kans	16.3	2.04	118	327	223
Indian Head, Saskatchewan	26.0	3.46	131	306	219
Moro, Oreg	20.2	3.31	109	301	205
Felt, Idaho	33.7	6.74	147	260	204
Mandan, N. Dak	25.7	3.09	122	269	196
Madison, Wis	26.1	3.84	110	271	191
Moccasin, Mont	19.5	3.33	105	265	185
Trebi:					
Aberdeen, Idaho	48.1	3.91	196	586	391
Madison, Wis	36.5	4.56	218	549	384
Indian Head, Saskatchewan	34.7	3.25	241	515	378
Bozeman, Mont	44.9	3.99	216	487	352
Hays, Kans	22.6	2.66	185	506	346
North Platte, Nebr	26.5	3.08	204	479	342
Mandan, N. Dak	30.6	3.34	201	465	333
Felt, Idaho	44.2	4.24	197	455	326
Moccasin, Mont	26.2	2.45	212	428	320
Moro, Oreg	27.1	2.78	166	447	307
Standard error of a difference			25.2	48.9	39.6

TABLE 12.—*Yields of seed lots of 1 variety of barley and 3 varieties of wheat, produced at 4 experiment stations in 1929 and 1930, when grown at Madison, Wis., Moccasin, Mont., and Aberdeen, Idaho, in 1930 and 1931, and weight of 1,000 kernels of seed sown*

Variety and station where seed was produced	Year seed was produced	Weight of 1,000 kernels sown	Yield of grain per 16-foot row						
			Moccasin, Mont., 1930	Madison, Wis., 1930	Moccasin, Mont., 1931	Madison, Wis., 1931	Aberdeen, Idaho, 1931	Average (3 stations 1931)	Average (5 station years)
Trebi barley:		*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Aberdeen, Idaho	1929	48.1	196	586	150	330	512	331	355
	1930	43.8			170	342	495	336	
Indian Head, Saskatchewan	1929	34.7	241	515	126	303	503	311	338
	1930	42.7			173	350	532	352	
North Platte, Nebr	1929	26.5	204	479	131	301	435	289	310
	1930	31.8			139	337	525	334	
Moccasin, Mont	1929	26.2	212	428	125	315	492	311	314
	1930	29.8			131	334	502	322	
Standard error of a difference			25.2	48.9	19.3	16.3	35	35.6	38.5

TABLE 12.—*Yields of seed lots of 1 variety of barley and 3 varieties of wheat, produced at 4 experiment stations in 1929 and 1930, when grown at Madison, Wis., Moccasin, Mont., and Aberdeen, Idaho, in 1930 and 1931, and weight of 1,000 kernels of seed sown*—Continued

Variety and station where seed was produced	Year seed was produced	Weight of 1,000 kernels sown	Yield of grain per 16-foot row						
			Moccasin, Mont., 1930	Madison, Wis., 1930	Moccasin, Mont., 1931	Madison, Wis., 1931	Aberdeen, Idaho, 1931	Average (3 stations 1931)	Average (5 station years)
Hard Federation wheat:		*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Indian Head, Saskatchewan	1929	34.8	151	100	99	100	420	206	174
	1930	27.1			79	101	388	189	
Aberdeen, Idaho	1929	39.8	147	104	115	91	362	189	164
	1930	40.3			107	97	457	220	
Moccasin, Mont	1929	24.2	129	85	85	72	377	178	150
	1930	32.4			100	116	443	220	
North Platte, Nebr	1929	18.8	132	86	93	74	365	177	150
	1930	17.5			97	80	447	208	
Peliss selection 89 wheat:									
Moccasin, Mont	1929	29.7	176	309	131	116	448	232	236
	1930	42.5			142	110	410	221	
Indian Head, Saskatchewan	1929	52.4	179	225	119	135	440	231	220
	1930	51.2			128	116	462	235	
Aberdeen, Idaho	1929	61.1	180	269			420		
	1930	58.3			125	122	438	228	
North Platte, Nebr	1929	39.1	171	214	103	156	400	220	209
	1930	28.5			103	118	435	219	
Marquis wheat:									
Aberdeen, Idaho	1929	35.1	129	179	91	82	450	208	186
	1930	35.3			115	99	392	202	
Indian Head, Saskatchewan	1929	29.0	136	178	96	87	388	190	177
	1930	27.1			91	96	352	180	
North Platte, Nebr	1929	19.4	123	145	81	92	435	203	175
	1930	17.0			93	92	338	174	
Moccasin, Mont.[1 2]	1929	18.0	139	179	75	84	373	177	170
Do.[1 3]	1929	16.3	163	138	103	94	373	190	174
Do.[1 4]	1929	15.0	162	156	89	96	400	195	181
Do.[1 5]	1929	9.0	151	120	83	81	400	188	167
Do.[1 6]	1930	22.3			83	85	333	167	
Do.[1 7]	1930	21.0			97		363		
Do.[1 8]	1930	9.2			84	74	320	159	
Do.[1 9]	1930	5.5			84	80	258	141	
Standard error of a difference			19.3	28.2	16.3	7.4	47.4	29.7	28.2

[1] Heads were tagged on July 16, 1929, and July 8, 1930, when anthers were protruded from florets near the middle of the heads, and harvested on the dates indicated.
[2] Mature stage.
[3] Hard-dough stage.
[4] Soft-dough stage.
[5] Early milk stage.
[6] Aug. 10.
[7] Seed sown at Moccasin and Aberdeen; harvested Aug. 1 and July 28, respectively.
[8] Seed sown at Moccasin, Madison, and Aberdeen; harvested July 19, 17, and 18, respectively.
[9] Seed sown at Moccasin, Madison, and Aberdeen; harvested July 16, 15, and 14, respectively.

Only 1 of the 57 seed lots of wheat and 2 of the 34 seed lots of barley gave yields that differed by more than twice the standard error from any other lot of the same variety. Seed of Peliss selection 89 produced at Moccasin in 1929 yielded more than seed from Hays, Mandan, and North Platte by as much as twice the standard error of the difference when only the average yields from the two tests in 1930 are considered. However, the seed from Moccasin and North Platte was included in the three tests in 1931, and the average yields reported in table 13 do not differ significantly. The yield of Marquis harvested on July 14, 15, and 16, 1930, when the weight of 1,000 kernels was only 5.5 g, was less than that of the highest yielding seed lot, which was produced at Aberdeen in 1929, the difference being only 2.3 times the standard error. Seed of Oderbrucker barley from Bozeman in 1929, yielded

more than seed produced at Moccasin by 2.2 times the standard error, and the difference between the yield of Trebi from Aberdeen and Moro in 1929, was 2.1 times the standard error. The seed of Oderbrucker from Bozeman and of Trebi from Aberdeen each weighed more per 1,000 kernels than other seed lots of the same variety. Differences in yield other than for the three seed lots mentioned are no greater than can be explained by random variation, and possibly these three should not be considered statistically significant inasmuch as the differences for the Marquis samples are based on yield tests at only three stations in 1931 and those for Trebi and Oderbrucker on yield tests at only two stations in 1930. It would be expected that in testing 91 seed lots some would be found which differ by more than twice the standard error owing to random variation alone.

Correlation coefficients were calculated for the weight of 1,000 kernels of the seed sown and the average grain yield, and for the weight of 1,000 kernels sown and dry weight of the seedlings at about the fifth-leaf stage. Correlation coefficients were first calculated for each variety, and since no significant differences were shown they were calculated for all varieties taken as a whole. The correlation coefficients are given in table 13.

TABLE 13.—*Coefficients of correlation for weight of 1,000 kernels sown and dry weight of 100 seedlings and yield of grain of wheat and barley varieties*

Year and variety tested for yield	Seed lots	Weight of 1,000 kernels sown correlated with—			
		Dry weight of 100 seedlings		Yield of grain	
		Correlation coefficient	*P*	Correlation coefficient	*P*
1930	*Number*				
Hard Federation	10	+0.8422	<0.01	+0.6082	0.07
Peliss selection 89	9	+.8801	<.01	+.1545	.69
Marquis	13	+.9797	<.01	+.4685	.11
Illinois No. 1	10	+.8986	<.01	+.2637	.46
4 varieties of wheat	42	+.9312	<.01	+.4026	.02
Hannchen selection 1	10	+.8201	<.01	+.1166	.75
Oderbrucker	10	+.8241	<.01	+.5521	.10
Trebi	10	+.8197	<.01	+.5004	.15
3 varieties of barley	30	+.8213	<.01	+.4049	.05
1931					
Trebi	8			+.7093	.05
Hard Federation	8			+.4498	.27
Peliss selection 89	7			+.4400	.33
Marquis	13			+.6535	.02
4 varieties	36			+.5978	<.01

The correlation coefficient for size of seed sown and grain yield was $+0.4026$, $P=0.02$ for wheat and $+0.4049$, $P=0.05$ for barley. Correlation coefficients for size of seed sown and yield were also calculated for the average yield at three stations in 1931 of one variety of barley and three varieties of wheat. Only seed lots from the stations which gave the maximum differences in yield in 1930 were included in these tests and, as would be expected, a higher correlation coefficient ($+0.5978$, $P<0.01$) was obtained.

The correlation coefficient for weight of seed and dry weight of seedlings for the four varieties of wheat was $+0.9312$, $P<0.01$, and for the three varieties of barley $+0.8213$, $P<0.01$. As would be

expected, the correlation is much greater between size of seed sown and dry weight of seedlings than between size of seed sown and grain yield. The effects of size of seed on vigor diminish as the plant approaches heading and maturity.

DISCUSSION

It has been shown that the environmental conditions under which seed of a variety of wheat or barley is produced may markedly affect the reaction of plants grown from it to seedling blight caused by *Gibberella saubinetii*. Seedling blight reaction was found to be influenced somewhat by protein content and size of seed sown. The data indicate that size of seed may have a slight influence on reaction to bunt caused by *Tilletia levis*. The effect on grain yield (when about equal numbers of kernels were seeded per row) was not pronounced, although differences of more than twice the standard error were obtained and a correlation between size of seed sown and yield was shown.

Such differences are not due to changes in the hereditary factors of the plant (i. e., the genes) but are simply differences in the expression of these factors resulting from environment. The optimum for each environmental factor for plant growth, such as light or temperature, varies as the other factors change, and the result is a complex interaction of all the factors that govern the expression of plant characters. In addition to the interaction of all the environmental factors at any stage of development, the physiological balance in the plant carried over from the preceding growth phase or from the embryo may influence development. The effect of environment on the seed while it is attached to the mother plant may be expressed in the developing seedling as a result of variation in the nutrients from the endosperm or as a direct result of variations in the reserve food and physiologic balance in the embryo. Maturation of seed is relative insofar as the condition of the embryo and endosperm is concerned, and, in wheat grown in the field, may take place as soon as 3 weeks or as late as 9 weeks after fertilization. It is a dormant condition of the embryo brought about by desiccation and is not definite as to the stage of development of the embryo or endosperm. Deming and Robertson (*2*) have shown that in the seed of some varieties a rest period after harvest is necessary before growth can be resumed but that in other varieties growth can be resumed immediately after or even before translocation of materials from the mother plant to the seed has ceased. In the small grains, dormancy during the period between germination and maturity is usually not so complete as is the dormancy of the seed, but here again the difference is only relative, as winter wheat plants thoroughly hardened during periods of low temperature respire very slowly. On the other hand, the respiration of seeds may be very slow under conditions of extreme desiccation and low temperatures, or may be very rapid under conditions favorable for germination. It would seem, then, that the effect on the subsequent crop of the environment under which seed is produced is directly comparable to the effect of environment during one stage of plant growth on subsequent stages; and it is generally recognized that plants transplanted to a widely different environment will not respond in the same manner as plants grown continuously in the same environment.

Dickson (*4*) has shown that the *Gibberella* parasite enters the seedling plant through natural wounds, at root ruptures, by means of mechanical injuries, and by direct penetration of the cortical tissues, as well as through the endodermis into the central cylinder of roots. In resistant plants, a suberinlike substance is deposited in the walls of cells around these ruptured tissues and the outer cortical cells, which retards or prevents penetration of the fungus. Infection takes place in wheat and barley seedlings prior to or very soon after emergence. It might be expected that a vigorous seedling could develop these protective barriers, which would enable it to resist invasion more effectively than would the slower growing seedlings of the same variety. Partial correlations show that size of seed influenced the reaction of the seedlings to seedling blight in seed lots produced in 1930 but not in 1929, and that it had more influence on seed lots of barley than of wheat. In fact, a negative correlation was obtained between size of seed sown and resistance to seedling blight in Hard Federation. It seems probable, however, that the true association is not between small seed and resistance but rather between resistance and some condition associated with small seed. The nature of this condition is not apparent from the present studies. The fact that plants from seed harvested 11 days after flowering at Moccasin, Mont., in 2 successive years had the same reaction to seedling blight as plants from mature seed and the further fact that plants from Madison-grown seed, even though it was shriveled, were infected less than plants from plump, well-developed seed from other stations indicate that the stage of development of the embryo or endosperm in the seed is not of great importance in determining the reaction of wheat to seedling blight caused by *Gibberella saubinetii*.

In view of the fact that size of seed and vigor of seedling growth account for only part of the differences in reaction to seedling blight of seed lots of the same variety, it would seem that a host-parasite relationship of nutritional nature might be partially responsible. This is in line with the work of Dickson (*3*, *4*), who has shown that the parasitic relation between *Gibberella saubinetii* and the seedlings of corn and wheat grown under different temperatures may be determined by differences in composition of the host plants. If host-parasite relationships can be altered so radically by a change in the composition of seedlings grown under different temperatures, it seems logical to expect these relationships to be altered as a result of differences in the chemical and physiological composition of the embryo and of the endosperm which furnishes food for its early growth.

The results of Dickson (*3*) show that wheat seedlings grown at low temperatures have relatively low nitrogen and high total sugar and dextrin content and are very resistant to seedling blight caused by *Gibberella saubinetii*. When grown at temperatures above 12° C. the nitrogen content increases and the total sugar and the dextrin content decrease and the seedlings are susceptible. The negative correlation found in the present studies between protein content of the seed lots and high indices of resistance to blight suggests a similar relation. While no data are available from the present study on the influence of chemical composition of the seed on composition of the resulting seedlings, it seems logical to expect that the balance between nitrogen and carbohydrates in the seed would be reflected in the germinating seedlings, and that seedlings from seeds relatively low in

nitrogen would be relatively high in carbohydrates, at least during the period when infection may occur.

The embryos in seeds of cereals (which are formed in the environment of the mother plant) contain the young plant with seminal roots, leaves, and the growing point of the axillary coleoptile tiller differentiated. Much evidence is available to show the variation that may occur in the chemical composition of the endosperm, which sustains the seedling during the first few days after germination, depending on the environment under which it was produced. The embryo in corn was shown by Hopkins et al. (*6*) to vary as widely in its chemical composition as the endosperm. Toole (*12*) has shown that the embryo starts growth before any changes take place in the endosperm, i. e., the embryo utilizes its own food reserve in starting growth. The seedling could, therefore, be in an active growing state before it was influenced by outside nutritional conditions even from the endosperm.

While the present studies are not conclusive, they do suggest that reaction to seedling blight of the seed lots of a variety grown under different environments is determined largely by a host-parasite relationship of a nutritional or biochemical nature rather than by differences in the morphological development of the embryo or size of the endosperm. Yield of grain and reaction to bunt may also be influenced by chemical and physiological composition as well as by size of seed sown, but the correlation between size of seed sown and these characters would seem to account for most of the differences observed in the present study.

The results indicate the importance of carefully selecting seed for conducting varietal tests for resistance, at least to some diseases. Differences in the source of the seed resulted in greater differences in reaction to seedling blight than were obtained between varieties. Similar results are more likely to be expected for characters that are expressed during the seedling stage than for characters expressed later in the life of the plant.

SUMMARY

Seed of four varieties of spring wheat and three of spring barley produced at several experiment stations in the United States and Canada in 1929 and 1930 was tested for reaction to seedling blight in the greenhouse at Madison, Wis., and for reaction to bunt and for yield in field trials at Moccasin, Mont., Madison, Wis., and Aberdeen, Idaho.

Environmental conditions under which the seed was produced affected the reaction to seedling blight (*Gibberella saubinetii*) of the subsequent crop. A relationship was found between low protein in the seed and high resistance indices and in some cases between large seed and high resistance indices, but these factors did not account for all differences. The results suggested that differences of a nutritional or biochemical nature were more important than morphological or size differences in the embryo and endosperm. Differences in disease reaction between seed of the same variety produced under different environmental conditions were greater than differences between varieties from seed produced at the same station. These results emphasize the necessity of carefully selecting seed for testing varietal resistance.

The environmental conditions under which the wheat seed was produced also affected the reaction to bunt, but the effects were much less pronounced than in the case of seedling blight. Only 1 of 76 seed

lots tested was considered to differ significantly from other seed lots of the same variety. However, a small correlation (-0.3036, $P=0.01$) was found between size of seed and reaction to bunt, the larger seed producing a lower percentage of infected plants.

Only 3 of the 91 individual seed lots produced under different environmental conditions differed significantly from other seed lots of the same variety in capacity to yield when a difference twice its standard error was considered as significant. Statistically significant correlation coefficients were obtained, however, for weight of 1,000 kernels sown and dry weight of 100 seedlings at about the fifth-leaf stage ($+0.9312$, $P<0.01$ for wheat and $+0.8213$, $P<0.01$ for barley) and for weight of 1,000 kernels sown and yield of grain ($+0.4026$, $P=0.02$ for wheat and $+0.4049$, $P=0.05$ for barley).

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VARIABILITY OF FIBER LENGTH IN A RELATIVELY UNIFORM STRAIN OF COTTON[1]

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INTRODUCTION

The general conception of a uniform sample of cotton (*Gossypium*) is one in which a large proportion of the fibers are the same length. With few exceptions, cotton breeders and other investigators who work with seed cotton have used the combing or butterfly[3] measurement of the fibers on a seed as an expression of lint length. The combing length is usually considered to be the modal length, although the actual mode can be determined only by some form of fiber sorting. The standard deviation of the combing lengths has been frequently used as a measure of the variability of the sample.

Continued selection of plants or progenies with low standard deviations of combing lengths may tend to reduce the total variation in lint length, but the investigator who is familiar with the natural diversity in the length of fibers on individual seeds will appreciate the limitations on progress toward uniformity by this method. This paper describes the methods used in determining the variability of length and gives the results of fiber-length studies of a strain of Pima cotton (*Gossypium barbadense* L.) grown at Sacaton, Ariz., in 1934.

TYPES OF COTTON FIBER SORTERS

A sorter for determining the length variability of ginned samples of cotton has been described by Webb (*6*).[4] Small pulls of paralleled fibers are drawn from the machine and laid out in the decreasing order of their lengths on boards covered with velvet. Each pull is measured and recorded in its proper length class. The class range is usually one-sixteenth or one-eighth of an inch, depending on the detail desired. The weight of the fiber is substituted for the frequency in calculating the variability of the sample, because of the impracticability of counting the individual fibers in each class.

Pressley (*4*) developed a machine for the sorting of cotton fibers from the seed. The combs in this sorter are one-eighth of an inch apart, and in addition to their function of holding the fibers in a parallel position, they give a measure of the fiber length. Another device for sorting fibers from the seed has recently been announced by McNamara and Stutts (*3*). The combs on this machine also are one-eighth of an inch apart. A sorter of this type was used in making the fiber arrays reported in this paper.

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[2] The writers are indebted to G. N. Collins, of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, for valuable advice concerning the application of the analysis of variance to their problem, and to E. Gordon Smith, of the Division of Cotton and Other Fiber Crops and Diseases, for aid in making the fiber combings and arrays.

[3] After the fibers on a seed have been straightened by combing, the distance from the point of attachment to the point where the fibers become noticeably scant may be called the combing length.

[4] Reference is made by number (italic) to Literature Cited, p. 762.

COMPARISON OF METHODS OF MEASUREMENT

Preliminary work on fiber arrays in which the several pulls were laid out on velvet-covered boards, measured, and then grouped and weighed has shown this to be a rather long and tedious procedure. According to the method reported by Pressley (*4*), all the fibers that occur between a given pair of combs are drawn out and weighed. Since the combs are spaced at regular intervals of one-eighth of an inch, they are used as the measure of the length of the fiber taken between each succeeding pair of combs. The method of actually measuring each pull with a ruler was compared with the method in which the combs of the McNamara sorter were used as the measure. By carefully laying out each pull and making proper notations of the pulls that occurred between each succeeding pair of combs in the sorter, both methods were compared on each fiber array. After each pull was measured, instead of weighing the combined pulls in a length class, as is the normal practice, each pull was weighed separately. One grouping of weights was made into the length classes as determined by the ruler and another grouping into the length classes as determined by the number of pulls between each sorter comb. Twenty single-seed samples were handled in this manner, and the mean length and variance of the fibers on each seed were calculated.

An average of the mean lengths showed that the comb method gave a slightly longer measurement than the ruler method, the mean difference, expressed as a fraction of a class length, being 0.1425 ± 0.0479, or 0.0178 inch. The probability of the occurrence of a fortuitous deviation as great or greater than the designated one is 27 in 10,000. However, the chances are 99 to 1 that the difference will be less than one sixty-fourth of an inch. While the odds seem rather high for the observed difference, the actual deviation is much smaller than could be detected by the method of measurement, and since neither method can be considered a standard it was assumed that either could be used as a measure of the length if both gave the same measure of variability of the fiber on the seed.

A comparison of the variance of the fiber on the seed, calculated from data obtained by both the ruler and comb methods, showed that a difference as large as one-seventieth of an inch would occur only once in 100 times. These findings were the basis for the adoption of the comb method of measurement for subsequent studies in fiber distribution.

CONSOLIDATION OF SUBSTAPLE LENGTH CLASSES

The proportion of fibers shorter than three-fourths of an inch in these samples was about 5 percent. Since this substaple is considered by spinners to be of doubtful value and information regarding its length distribution was not needed, it seemed practical to consolidate all of these shorter lengths into one class, thus reducing further the time and labor of arraying a sample of cotton. Using the same arrays described in the preceding section, the weights of all length classes under three-fourths of an inch were grouped together and treated as one class. When these arrays so grouped were compared with arrays of the same samples in which the fibers were placed in $\frac{1}{8}$-inch groups ranging from the longest to the shortest, the procedure by which the length classes under three-fourths of an inch were combined gave a difference of mean lengths which was 0.0175 ± 0.0122 of a class length longer than these same arrays when the shorter lengths were not combined. The probability of a fortuitous occurrence of a deviation of this size is 16 in 100.

DIFFERENCES IN MEAN LENGTH OF FIBERS FROM RIGHT AND LEFT SIDES OF SEED

In the preparation of the seed-cotton samples for sorting, the method outlined by Cook (*1*) for parting the fibers on the raphe or seam of the seed was followed (figs. 1 and 2). The same methods of preparation and sorting were applied to each seed. When the seed on which the fiber had been parted was placed with the raphe side up and the tip or chalazal end pointed toward the operator, the side of the seed on the operator's right was designated as the right side and the other side was designated as the left. It was possible, by a comparison of the mean lengths (as determined from the fiber-array analysis) of the fiber from the right side of a seed with that from the left side, to determine the differences in fiber length between the two sides of the seed. Using the method of sorting previously described, the fibers from the right and left sides of seeds from four samples per plant from 19 plants were arrayed. The mean lengths of the fibers from the right side of the seed averaged 0.6919 ± 0.1023 of a class length, or 0.0865 inch longer than the means of the left side. The significance of this difference is emphasized by calculating the maximum difference that could be expected to occur by chance once in 100 times, which in this case was found to be 0.033 inch.

Whether this difference in mean length of the fibers on the right and the left side of the seed is inherent or is due to the technique of the operator was not determined. However, the fact is that measurable differences were observed when methods that seemed most practical for this work were used, and in view of these findings it seems that the proper expression of the mean length of the fibers on a seed may be obtained only by sorting the fibers combed out on both sides of the seed. Because of this necessity it was impossible to get two strictly comparable measures of length from a single seed and thus calculate the experimental error of the method of determination of the distribution of the fibers on a single seed.

CALCULATION OF MEAN LENGTH AND STANDARD DEVIATION OF FIBERS FROM INDIVIDUAL SEEDS

The calculation of the mean length, standard deviation, etc., of an array of fibers from a cottonseed used in this experiment followed the familiar method where the assumed mean, 0, is used in determining the squared departures. The length classes were expressed in ⅛-inch units; the weight of the fibers in each class (to the nearest 0.02 mg) was substituted for the number of fibers as the frequency.

When l = length of a class
w = weight of fibers in a class
$W = \Sigma w$

the various formulae are as follows:

$$\text{Mean} = \frac{\Sigma lw}{W}$$

$$\sigma^2 = \frac{\Sigma l^2 w - \frac{(\Sigma lw)^2}{W}}{W-1}$$

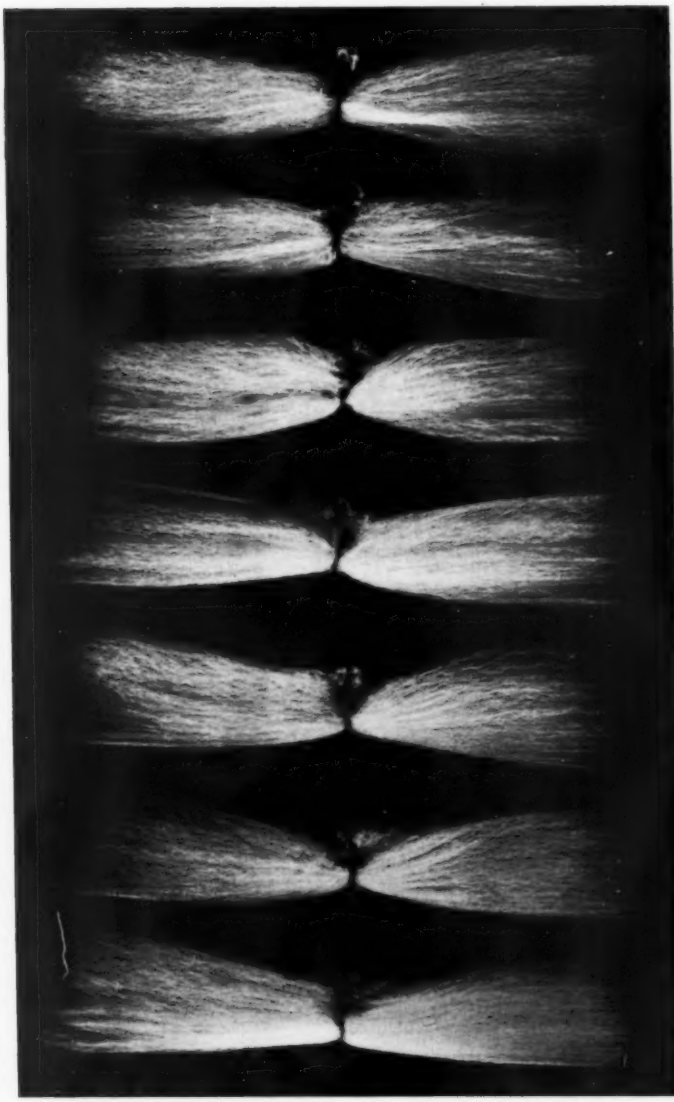

FIGURE 1.—Combings from random samples from consecutive Pima cotton plants, showing relative uniformity of the combing lengths. Natural size.

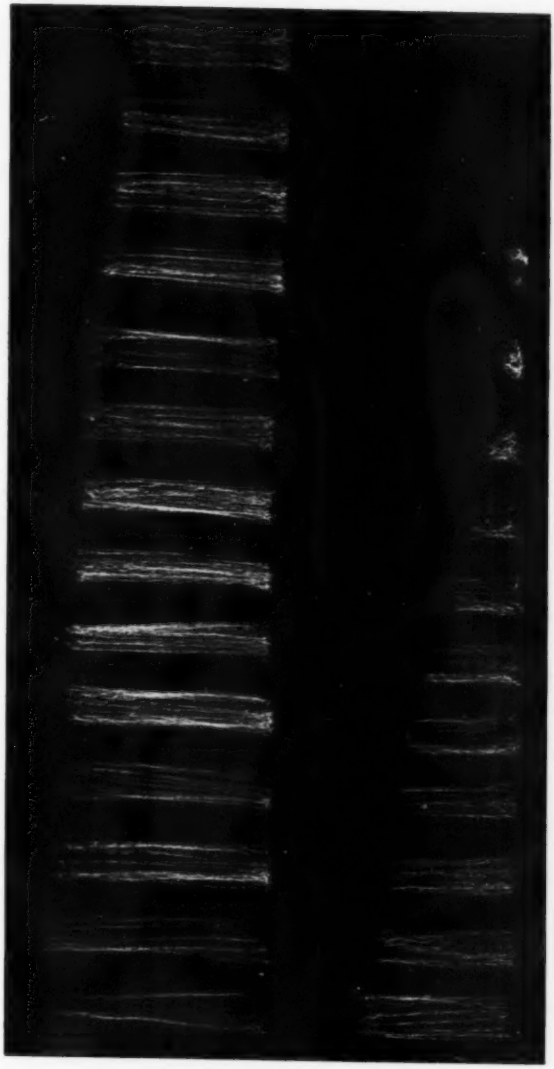

FIGURE 2.—Fiber array, showing the variation in length of fibers from a single seed. Three-quarters natural size.

The data secured from the fourth sample from Pima plant no. 4 follow:

l	w
14	0.58
13	2.78
12	6.86
11	7.38
10	4.86
9	3.94
8	3.24
7	2.28
6	1.38
4 [1]	3.80
	37.10

[1] Classes 5 to 1 (less than 3/4 inch) are consolidated.

When the formulae just given are applied to these data, the results are as follows:

$$\frac{\Sigma lw}{W}=\frac{357.18}{37.10}=9.627\ (=1.204\text{ in.})=M.$$

$$\Sigma l^2w=3{,}699.020$$

$$\frac{(\Sigma lw)^2}{W}=\frac{3{,}438.748}{260.272}$$

$$\frac{260.272}{(37.10-1)}=7.2098=\sigma^2$$

$$2.6851=\sigma$$

VARIATION IN FIBER LENGTH DUE TO POSITION IN THE LOCK

A lock of cotton is composed of several seeds held together by the natural entwining and intermingling of the fibers. Extreme care must be exercised if a seed is to be disengaged without detaching a considerable amount of fiber. It was found to be impracticable to separate the seeds of a lock in such a way that individual seeds could be taken at random. Therefore, the lock, which is the natural unit in seed cotton, was taken at random. In order to prevent discrimination in sampling, it was necessary to determine the amount of variation in mean length of the fibers on individual seeds within the lock that was due to the position of the seed in the lock. Two locks were taken at random from the bulked cotton from each of 19 consecutive plants. A seed from the bottom, middle, and top positions was taken from each lock, and fiber arrays were made of each. The mean length of the fibers on each seed was determined, an analysis of the variance of which is given in table 1. It will be noted that F, as derived by the method outlined by Snedecor (*5*) for means of positions, is well under the F value for a probability of 0.01.[5] From the standard error as calculated from the remainder variance, the chances are found to be 99 to 1 that the difference in mean lengths of the fibers among the three positions is less than one thirty-second of an inch. The F value found in the analysis in table 1 indicates that the samples from the three positions in the lock used in this study may be considered

[5] The significance of F, which is an expression of the relation of the larger mean square to the smaller, is influenced by the number of degrees of freedom in the two mean squares. Tables have been computed which give the F values, for appropriate degrees of freedom, for probabilities of 0.05 and 0.01.

homogeneous in mean fiber lengths, and this would ordinarily preclude further comparisons in which the error of the difference of part of the data was used (7).

TABLE 1.—*Analysis of variance of mean lengths of fibers on individual seeds from top, middle, and bottom positions in each of two locks per plant from 19 plants of Pima cotton*

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	113	51.4997	0.4557
Within classes [1]	57	25.7921	.4525
Means of plants	18	21.6540	1.2030
Means of positions [2]	2	.6851	.3426
Remainder	36	3.3685	.0936

[1] This portion of the analysis is the variance of lock positions on single plants, and is not to be confused with "means of positions", which is the variance among positions from all plants.

[2] For means of positions, $F=\frac{0.3426}{0.0936}=3.66$. For a probability of 0.01, F would be 5.27.

The following data show comparisons of the average of the mean fiber lengths on seeds from top, middle, and bottom positions in locks from two locks from each of 19 plants.

Position in locks		Fiber length (M±S. E.)
Middle	class lengths [1]	9.815±0.0988
Bottom	do	9.631± .1218
Difference	do	.184± .1568
Do	inch	.023± .0196
D/E	ratio	1.17
Middle	class lengths	9.815± .0988
Top	do	9.733± .1076
Difference	do	.082± .1461
Do	inch	.010± .0183
D/E	ratio	0.56
Top	class lengths	9.733±0.1076
Bottom	do	9.631± .1218
Difference	do	.102± .1625
Do	inch	.013± .0203
D/E	ratio	0.63

[1] One class length=one-eighth of an inch.

Since the mean lengths of fibers from seeds within the three positions measured did not differ significantly, it was believed that a seed taken from any part of the lock would be representative, but as a precaution against the introduction of an error due to some position not being accounted for in this analysis, a seed from the middle of each lock (the lock having been taken at random) was used as the sample in subsequent studies.

RELATION OF THE MEAN TO OTHER METHODS OF MEASUREMENT

In this study the mean is used as the expression of the length of fibers on a seed. It is calculated in the usual manner by multiplying each class length by the weight of the fibers in that class and dividing the sum of lengths × weights by the sum of the weights. In addition

to the mean, Webb (*6*), in his work with the ginned lint, calculated the length at the upper quartile. This is the length of the group at that point on the array which includes 75 percent by weight of the fibers, beginning with the shortest groups. From the data given in the example on page 754, the upper quartile length is calculated as follows:

Find 25 percent of the total weight of the sample:

$$37.10 \text{ mg} \times 0.25 = 9.275 \text{ mg}$$

Find the number of classes, beginning with the longest, the sums of the weights in which will come nearest to equaling $\Sigma w \times 0.25$ but will not exceed it. Classes 14 and 13 may be summed, but the addition of class 12 brings the total above 9.275 mg.

From 9.275 subtract 3.36 (Σ classes 14 and 13), leaving 5.915 mg, which is the amount of the upper quartile extending into class 12. Since class 12 contains 6.86 mg, the percentage of class 12 in the upper quartile may be calculated by dividing 5.915 by 6.86, which gives 0.8622. Subtract this factor from the length of the next higher class: $13 - 0.86 = 12.14$ (eighths of an inch), or 1.52 inches.

The combing length is the measurement in general use by cotton breeders. Although the method to be used for measuring may depend on the preference of the investigator, the usual method of preparing the sample is that reported in this paper. A system of grades together with an easy and convenient method of measuring the combing length of the cotton lint while attached to the seed has been developed by Kearney (*2*), and this method was used in determining the combing length of the samples used in this study. The average of the 76 determinations of mean fiber length was 9.872 class lengths of one-eighth of an inch each (=1.234 inches); the average of the upper quartile lengths was 1.519 inches; and the average combing length was 7.71 grades (=1.589 inches).

The correlation of the mean length with the upper quartile length and with the combing length, as determined from 4 samples from each of 19 consecutive plants, is given in table 2. Reference to this table shows that the correlation coefficient for the mean with combing-length measurement is +0.8049 and that for the mean with upper quartile length is +0.9518. In view of the very close relationship between these expressions of lint length, it is apparent that after one has been determined the others may be predicted with a considerable degree of accuracy.

TABLE 2.—*Analysis of variance and covariance of different methods of measuring fiber length, as determined on the fiber of 4 seeds from each of 19 plants of Pima cotton*

MEAN LENGTH AND COMBING LENGTH

Source of variation	Degrees of freedom	Mean lengths		Lengths		Covariance	
		Sum of squares	Mean square	Sum of squares	Mean square	Sum of products	Coefficient of correlation
Total	75	25.4175	0.3389	29.1316	0.3884	21.9010	+0.8049±0.0404
Means of plants	18	8.6328	.4796	10.0066	.5559	7.6970	+.8281± .0721
Samples on plant	57	16.7847	.2945	19.1250	.3355	14.2040	+.7928± .0488

MEAN LENGTH AND UPPER QUARTILE LENGTH

Total	75	25.4175	0.3389	0.5174	0.0069	3.4515	+0.9518±0.0108
Means of plants	18	8.6328	.4796	.1739	.0097	1.1842	+.9665± .0151
Samples on plant	57	16.7847	.2945	.3435	.0060	2.2673	+.9443± .0142

SOURCES OF VARIATION IN FIBER LENGTH

The development of a practical method of sorting lint from the seed offers opportunities for further investigation of the sources of the variation in fiber length. Four random samples (locks) were taken from each of 19 consecutive plants in a row of a commercial strain of Pima cotton growing in the yield test at Sacaton, Ariz., in 1934. In accordance with the procedure adopted, a seed from near the middle of each lock was taken, combings were made of each seed, and the combing length was graded. The fibers from each seed sample were sorted, and the mean length, the upper quartile length, and the variance were calculated. The total variance in mean lengths was apportioned between that which was due to variation among the means of different samples from the same plant and that which was due to variation among the means of plants in the row, as shown in table 3. The mean squares of the two sources of variation are so nearly equal that the samples must be considered as coming from a homogeneous population. The F value for means of plants is well below the F value for a probability of 0.01.

TABLE 3.—*Analysis of variance of mean lengths of fibers on individual seeds from 4 samples from each of 19 plants of Pima cotton*

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	75	24.4173	0.3256
Means of samples from the same plant	57	15.7846	.2769
Means of plants [1]	18	8.6327	.4796

[1] For means of plants, $F=\left(\frac{0.4796}{0.2769}\right)=1.73$. For a probability of 0.01, F would be 2.12.

An adaptation of the method of analysis of variance which expresses the extent to which variation of the lint on the seed contributes to the total variance of the composite sample has been suggested by G. N. Collins. The formulae and an example of this method, involving 2 samples per plant from the first 10 plants used in this experiment, follow.

To subdivide the variation in a composite sample into the portions contributed by (1) departures in the length of fibers from the mean of the seed on which they are borne; (2) departures in the mean lengths of fibers on the seeds from the mean of the plant; and (3) departures in the mean length of fibers on the individual plants from the general mean,

Let: W_s=weight of fibers on a seed,
W_p=weight of fibers on a plant,
W_t=total weight of fibers,
lW_s=sum of the products (length×weight) on a seed,
lW_p=sum of the products (length×weight) on a plant,
lW_t=sum of the products (length×weight) for entire population,
l^2W_s=sum of the products (squared length×weight) on a seed.

Then:

Sum of squared departures on the seed=

$$l^2W_s-\sum\frac{(lW_s)^2}{W_s} \qquad (1)$$

Sum of squared departures among means of sample on the same plant=

$$\sum \frac{(lW_s)^2}{W_s} - \sum \frac{(lW_p)^2}{W_p} \quad (2)$$

Sum of squared departures among means of plants=

$$\sum \frac{(lW_p)^2}{W_p} - \frac{(lW_t)^2}{W_t} \quad (3)$$

Table 4 shows data for fiber lengths on 2 seeds from each of 10 plants. By means of the formulae just given, the analysis of variance in table 5 was calculated from the data in table 4.

TABLE 4.—*Data from sorting of fibers from 2 seeds from each of 10 plants of Pima cotton*

Plant no.	Sample no.	W_s	W_p	lW_s	lW_p	l^2W_s	$\frac{(lW_s)^2}{W_s}$	$\frac{(lW_p)^2}{W_p}$
1	2	3	4	5	6	7	8	9
1	1	43.84	81.00	424.16	810.84	4,289.44	4,103.8254	8,116.8087
	2	37.16		386.68		4,242.32	4,023.7196	
2	1	33.20	71.66	336.06	692.82	3,563.22	3,401.6064	6,698.2913
	2	38.46		356.76		3,549.60	3,309.3525	
3	1	42.68	79.90	428.92	804.24	4,539.48	4,310.5053	8,095.1436
	2	37.22		375.32		3,996.76	3,784.6615	
4	1	39.80	79.44	382.42	791.10	3,926.38	3,674.4989	7,878.1371
	2	39.64		408.68		4,450.36	4,213.4041	
5	1	36.78	81.14	352.04	824.12	3,554.92	3,369.5530	8,370.3941
	2	44.36		472.08		5,248.56	5,023.8847	
6	1	35.02	69.68	325.52	660.24	3,201.16	3,025.7929	6,255.9825
	2	34.66		334.72		3,434.12	3,232.4719	
7	1	41.40	81.76	400.78	762.48	4,106.26	3,879.8214	7,110.7602
	2	40.36		361.70		3,417.70	3,241.4987	
8	1	35.38	79.02	377.96	829.06	4,251.56	4,037.6981	8,698.3103
	2	43.64		451.10		4,926.14	4,662.9516	
9	1	44.20	81.46	452.82	804.30	4,953.94	4,639.0486	7,941.3024
	2	37.26		351.48		3,527.20	3,315.5714	
10	1	44.78	74.78	415.74	725.70	4,097.06	3,859.7531	7,042.5313
	2	30.00		309.96		3,395.40	3,202.5067	
Total		779.84	779.84	7,704.90	7,704.90	80,671.58	76,312.1258	76,207.6615

Sum of squares on the seed, formula (1).—In table 4 the total of column 7 $=\Sigma l^2W_s=80{,}671.58$, and the total of column 8 $=\sum \frac{(lW_s)^2}{W_s}=$ 76,312.1258. The difference, 4,359.4542, is the sum of squares on the seed.

Sum of squares among means of samples on the plant, formula (2).—The first term of formula (2), $\sum \frac{(lW_s)^2}{W_s}$, is the total of column 8, and is 76,312.1258. To calculate the second term, the values in column 6 are squared and divided by the corresponding values in column 4. The sum of these quotients $=\sum \frac{(lW_p)^2}{W_p}=76{,}207.6615$. The first term minus the second term, $76{,}312.1258-76{,}207.6615=104.4643$, is the sum of squares among means of samples on the same plant.

Sum of squares among means of plants, formula (3).—The first term, $\sum \frac{(lW_p)^2}{W_p}$, as just calculated, is 76,207.6615. The second term, $\frac{(lW_t)^2}{W_t}$, is the total of column 5, squared and divided by the total of column 3, and is 76,125.2103. The first term minus the second, $76{,}207.6615-76{,}125.2103=82.4512$, is the sum of squares among means of plants.

Total sum of squares.—It will be noted that the correction term in each formula becomes the first term in the succeeding formula. Thus the first term in formula (1) minus the correction term in formula (3), 80,671.58 − 76,125.2103 = 4,546.3697 = the sum of three variances and is the total variance.

In calculating the degrees of freedom the grand total of the weights used as the frequencies is rounded off to the nearest milligram for the final divisions in table 5 (sum of squares ÷ degrees of freedom = mean square). Thus for the total there were 780 − 1, or 779; on the seeds, 1 degree of freedom on each of the 20 arrays was sacrificed, leaving 780 − 20, or 760; among means of samples from the same plant there were 2 samples per plant with 1 degree of freedom per plant, or 10 for the 10 plants; and among the means of plants there were 10 − 1, or 9.

The analysis of variance of the 76 seed samples previously discussed is given in table 5. This table shows substantial agreement with the length measurements which were taken on mean lengths alone, as given in table 4. There was no significant difference in the mean square determinations between two samples from each of 10 plants (table 5, column 4) and four samples from each of 19 plants (table 5, column 7). The variation among means of plants is no greater than might be expected from the variability of samples from the same plant. Compared with the variation of fibers on the seed, however, the variability of plant means appears significant. The latter comparison indicates that differences among means of plants were detected and that this variance, although an insignificant part of the total, was larger than was expected from a random sample having the variance of the fibers on individual seeds. Even with complete genetic uniformity, variations in plant means are to be expected as a result of variations in environment. In the present instance the differences in plant means undoubtedly would have been greater had the plants been selected from scattered areas in the field.

TABLE 5.—*Analysis of variance of fiber length in composite samples of fiber from plants of Pima cotton*

Source of variation	Fibers from 2 seeds from each of 10 plants			Fibers from 4 seeds from each of 19 plants		
	Degrees of freedom	Sum of squares	Mean square	Degrees of freedom	Sum of squares	Mean square
Total	779	4, 546. 3697	5. 8362	2, 779	17, 516. 1269	6. 3030
On seeds	760	4, 359. 4542	5. 7361	2, 704	16, 673. 1265	6. 1661
Means of samples from the same plant	10	104. 4643	10. 4464	57	551. 1960	9. 6701
Means of plants	9	82. 4512	9. 1612	18	291. 8044	16. 2114

Comparison of mean squares	Fibers from 2 seeds from each of 10 plants		Fibers from 4 seeds from each of 19 plants	
	Value of F	Value of F for P=0.01	Value of F	Value of F for P=0.01
Means of samples from the same plant / On seeds	1. 82	2. 25	1. 57	1. 79−
Means of samples from the same plant (or reciprocal) / Means of plants	1. 14	4. 31	1. 68	2. 50
Means of plants / On seeds	1. 59	2. 53	2. 63	1. 80

The standard deviation of fibers on the seed (2.4802) is only 1.1 percent less than the observed standard deviation of the entire composite sample (2.5106). This may be taken to indicate that if there were no differences in the true means of fiber length among samples from the same plant or among means of plants the variation of the composite sample would be reduced 1.1 percent. In other words, these data show that in this sample of Pima cotton more than 98 percent of the variability in lint length as measured by the standard deviation was due to the variation of the fibers on the seed. The amount of variation contributed by the fibers on the seed is further emphasized in figures 1 and 2. The combings shown in figure 1 were made from random samples from consecutive plants; figure 2 shows a fiber array made from a representative seed. It is obvious that the

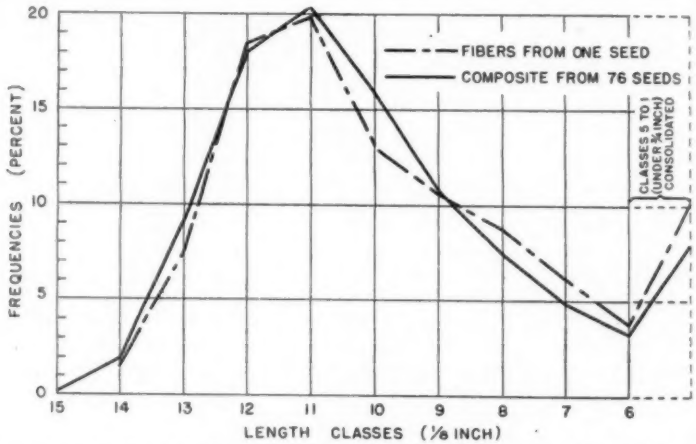

FIGURE 3.—Comparison of the frequency distribution of lengths of the fibers on a single representative seed of Pima cotton with that of a composite sample of the fibers from four seeds from each of 19 Pima plants.

variation in combing lengths in figure 1 is very much less than that of the fibers from the individual seed as shown in figure 2. The same idea is brought out graphically in figure 3. The solid line represents a composite array of 76 individual seed samples, and the broken line represents the distribution of fiber lengths on a single seed. Although in both graphs the class lengths are exaggerated, the cumulative curves in figure 4 show, even more strikingly than the curves in figure 3, the similarity between the frequency distributions of the fibers from 1 seed and from 76 seeds, respectively.

DISCUSSION AND CONCLUSIONS

The fiber-sorting methods developed in this study were incidental to the analysis of variance of the fiber lengths in a variety of cotton. In Pima cotton, the variety used in this study, the general plant characters have been rather definitely fixed through many years of inbreeding followed by careful roguing each year after the seed distribution reached commercial proportions. Since the samples were

obtained from Pima cotton plants that were normal in appearance, having been cultivated and irrigated in accordance with accepted practices, and from a small section of a row in which the plants were uniform in growth, it was not surprising that there was little variation either among the means of different samples from a single plant or among the means of plants in the row. The results obtained in this study show that the variation in fiber length on seeds was responsible for as much as 98 percent of the total variation in the composite sample and indicate that if further reduction in the total variance of the fiber lengths in composite samples of Pima cotton is desired the most rapid approach would be by breeding for a reduction of the variance of the fiber lengths on seeds.

The general plant and fiber characters in some varieties may not be as well fixed as in Pima. Combing length measurements of seed samples of cotton from mixed or deteriorated seed stocks have often given a wide range of differences. Obviously, the total variance in

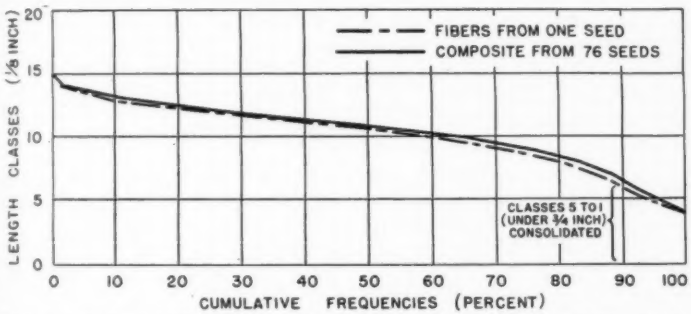

FIGURE 4.—Ogive curves comparing an array of fibers from a single representative seed of Pima cotton with that of a composite sample of the fibers from 4 seeds from each of 19 Pima plants.

fiber length among consecutive plants grown from such a seed stock would be geater than that which was found in Pima cotton, but even in extremely diverse stocks the variability of the fibers on individual seeds would contribute a large proportion of the total variance.

The extent or intimacy of the mixture of the fibers from different seeds, which make up the ginned sample, has not been determined, but it is believed that the mixture is thorough enough to be considered a representative sample of the fibers from the individual seeds. When composite or ginned samples are used, variations in the fiber length contributed by individual plants cannot be detected and plants with wide differences in mean lengths of fiber may make a composite fiber array, the mean length of which would be little different from that of a uniform variety. The dispersion of the fibers undoubtedly would be greater in the mixed sample, as would be the usual case where cottons become mixed under commercial conditions. Sorting of seed-cotton samples affords a method by which the variation of fibers on seeds can be studied, and the application of the analysis of variance makes it possible to express this variation as its proportionate part of the total.

The cotton breeder has been confused by the broad usage of the term "fiber-length uniformity." Two schools of thought, each with numerous variations, may be recognized. One, which is supported by

the majority of spinners, cotton technologists, and cotton breeders, thinks of uniform cotton as a sample in which all of the fibers tend to approach one length. The other group, which seems to be in the minority at present, thinks of a uniform sample of cotton as one made up of fibers from seeds which, if combed and sorted while attached to the seeds, would have had similar means, modes, and standard deviations. No attempt has been made in this study to determine which kind of uniformity is desired by the spinners, but from the standpoint of the cotton breeder there is an urgent need for a decision on this question. If the first idea proves to be correct, plant selection in well-bred varieties to increase the uniformity of mean fiber length hardly can be expected to produce any reasonable increase in the fiber uniformity of the composite or ginned sample. Considerable improvement in fiber uniformity of this type may be expected by selecting plants with low standard deviations of lint length as determined from fiber arrays of seed cotton. If the other kind of uniformity is desired, selection on the basis of plant means would, of course, be the proper procedure; but it would seem that thorough mixing of the fibers of stocks that vary somewhat in plant means would be equally advantageous. The second conception of fiber uniformity would obviate the necessity of sorting the fiber on individual seeds and calculating its variability.

SUMMARY

With samples from a commercial strain of Pima cotton, fiber-sorting methods were developed which materially reduced the time required for making fiber arrays of cottons of the Egyptian type.

The mean and the upper quartile length, which were determined from fiber arrays from the same seeds, were found to be highly correlated, as were the mean and the combing length.

The differences from sample to sample on the plant and from plant to plant in the row were insignificant, and analysis of variance showed that the samples could be considered as coming from a homogeneous population.

Of the total variance in fiber length, as determined from sorting the fibers on the seed, the variance on the individual seeds contributed 98 percent, leaving the remainder attributable to that which occurred among the means of samples on the plant and among the means of plants in the row.

The recognition of the amount of variance that is due to the variation in the fiber length on the seed raises questions as to the definition of the term "uniform fiber length" and the methods to be followed by the cotton breeder in cotton improvement work.

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NITROGEN UTILIZATION BY OPHIOBOLUS GRAMINIS[1]

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INTRODUCTION

It is an established fact that there exists a specificity among microorganisms for the kind of nitrogen compound they will utilize in their metabolism. Some are able to utilize many kinds of these compounds, others only a few.

Most of the nutritional work on fungi has been done with saprophytes. It seems that more should be done with the parasites. A knowledge of the nutritional requirements of parasitic fungi should be of aid in interpreting the phenomena of their specificity for certain hosts and of the method of hyphal penetration into plant tissue as well as the establishment of the organism after penetration. The differences in virulence among different species as well as among strains of one organism might well depend on the ease with which the available food can be utilized.

The investigations herein reported were undertaken to determine to what extent *Ophiobolus graminis* Sacc., which causes take-all of wheat, is specific in its utilization of nitrogen from different sources. A brief summary of the results has been published elsewhere,[2] and it is the purpose of the present paper to give the complete report.

METHODS

Throughout this work a liquid modified Czapek's medium of the following formula was used:

Magnesium sulphate ($MgSO_4$)	grams	0.5
Potassium dihydrogen phosphate (KH_2PO_4)	do	1.0
Ferrous sulphate ($FeSO_4$)	do	.01
Sucrose or other carbohydrate	do	30.0
Nitrogen	Source and amount varied	
Water	cubic centimeters	1,000

The hydrogen-ion concentration of the medium was adjusted as nearly as possible to within a favorable range for the growth of *Ophiobolus graminis*. Pyrex flasks of 125-cc capacity were used as the receptacles for the medium. Fifty cubic centimeters of the prepared medium was introduced into these flasks.

Prepared media were steam sterilized at 15 pounds' pressure for 20 minutes, except when the media contained compounds known to break down at high temperatures. In such cases sterilization was intermittent in the steam sterilizer.

[1] Received for publication July 28, 1936; issued January 1937. Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Department of Botany, Kansas Agricultural Experiment Station. Contribution no. 358, Department of Botany, Kansas Agricultural Experiment Station.

[2] FELLOWS, H. NITROGEN METABOLISM OF OPHIOBOLUS GRAMINIS. (Abstract) Phytopathology 26: 93. 1936.

Ophiobolus graminis was grown on potato-dextrose agar in Petri dishes. The agar with the adhering mycelium was cut into small blocks and one block was placed in each flask. The temperature of the growing cultures was maintained at 24° C. in an electric incubator. For the most part, growth observations were made by the unaided eye, and the relative abundance of growth was rated from none to the best by the symbols 0, 1, 2, 3, 4, and 5. In a few instances the mycelium was dried and weighed.

The nitrogen sources used consisted of both inorganic and organic compounds, as nearly chemically pure as can be purchased on the market. The organic nitrogen compounds included proteins of various complexity, representative members of the aliphatic and aromatic nitrogen series, the alkaloids, and miscellaneous compounds.

Certain nitrogen compounds probably are toxic to all forms of cryptogams. A pinch of garden soil was added to one flask of each type of culture to find whether any of the many organisms present would grow. Lack of all growth should be an indication of general toxicity. *Rhizopus* sp. and *Penicillium* sp., two vigorous growth types, were sown in other flasks for the same purpose.

It was necessary to exercise caution concerning the concentration of certain compounds that were being tested for the first time in a nutrient solution. A concentration that would permit growth was attained by adding to the culture solution containing the untried source of nitrogen a small amount of casein, which is utilized by *Ophiobolus graminis*. Growth under these conditions was taken to indicate that the material was not toxic, or if so, that the concentration was not great enough to be injurious. Two grams of any of the compounds tried in 1,000 cc of the medium was found not to be too concentrated.

RESULTS

NITROGEN COMPOUNDS AS NUTRIENT SOURCES

Table 1 shows the ability of *Ophiobolus graminis*, *Rhizopus* sp., and *Penicillium* sp. and of organisms in garden soil to grow in cultures in which nitrogen was supplied from various sources. Of all compounds tried, egg albumen, casein, peptone, and nucleic acid were the only ones that supported a growth of *O. graminis*, although many of them supported at least some growth of *Rhizopus* and *Penicillium*. Acetanilide, diphenylamine, cyanamide, dicyandiamide, and benzylamine appeared to be toxic to all the forms of life that were introduced. Hippuric acid, benzenesulphonamid, and betaine hydrochloride were evidently poor sources of nitrogen for all of the many fungi and bacteria from the soil.

Since *Ophiobolus graminis* utilized egg albumen and casein, it appeared possible that it might utilize some of the hydrolytic products of these proteins. Six of seven of such hydrolytic products of casein and five of the seven from egg albumen were tried without success. These products were included in table 1. Growth was also negative when these products were mixed in the same proportion that they are found to be present by analysis in the respective proteins.

TABLE 1.—*Relative growth of Ophiobolus graminis, Rhizopus sp., Penicillium sp., and fungi and bacteria from garden soil in Czapek's solution with nitrogen from different sources*

Source of nitrogen	Tests	Relative growth [1] of—			
		Ophiobolus graminis	*Rhizopus* sp.	*Penicillium* sp.	Soil organisms
Inorganic:	*Number*				
Ammonium acetate	2	0			5 F B.
Ammonium carbonate	8	0	1	3	5 F.
Ammonium chloride	6	0	5	4	1 F.
Ammonium formate	2	0	3	4	5 F.
Ammonium nitrate	6	0	5	4	5 F.
Ammonium oxalate	6	0	1	5	5 F.
Ammonium phosphate, mono	7	0	5	4	5 B F.
Ammonium phosphomolybdate	6	0	5		
Ammonium sulphate	9	0	5	4	5 F.
Ammonium tartrate	4	0	5	4	5 F.
Calcium nitrate	2	0	0	5	5 B.
Magnesium nitrate	2	0	0	4	5 F.
Potassium nitrate	6	0			5 B F.
Sodium nitrate	5	0	5	5	5 F.
Sodium nitrite	2	0			5 F.
Organic:					
Acetamide	2	0	3	3	5 F.
Acetanilide	3	0			0.
d-alanine	2	0	5	2	5 F.
Albumen, egg	6	5			
Allantoin	2	0	5	2	5 B.
Asparagine	2	0	5	3	5 B.
Aspartic acid	2	0	5	3	5 B.
Benzenesulphonamid	2	0	0	0	T F.
Benzylamine	2	0	0	0	0.
Betaine hydrochloride	2	0	0	0	1 B F.
Caffeine	5	0	1		
Casein	Many	4			
Choline hydrochloride	2	0	2	3	5 B F.
Creatine hydrate	2	0	0	2	5 B.
Cyanamide	4	0			0.
Dicyandiamide	2	0	0	0	0.
Dinitrobenzoic acid	2	0	0	0	T F.
Diphenylamine	4	0			0.
Gelatin	2	0	3	2	3 B.
Glutamic acid	2	0	5	5	5 B.
Glycine	7	0	5	3	5 B.
Guanidine carbonate	2	0	0	4	5 B.
Hippuric acid	4	0			1
Hydroxylamine hydrochloride	2	0			3 F.
Leucine	2	0	5	2	5 F B.
Methylamine hydrochloride	2	0	2	2	5 B.
Nicotine	4	0	0		5 F.
Nucleic acid	2	2			
Peptone	5	5			
Phenylalanine	2	0	4	3	5 F.
Taurine	2	0	2	3	5 F B.
l-tyrosine	2	0	2	2	5 F.
Urea	2		50	4	5 F B.
Uric acid	4				5 F.
Control (no nitrogen)	Many	0	T		T.

[1] 0 indicates no growth, while 1 to 5 indicate 5 degrees of growth. F stands for fungi, B for bacteria, and T for trace.

EFFECT OF SOURCES OF CARBON

Dextrose, sucrose, maltose, starch, dextrin, lactose, *d*-fructose, and *l*-xylose were added separately to portions of several batches of media, each batch containing a different source of nitrogen. The nitrogen compounds were egg albumen, casein, peptone, sodium nitrate, ammonium chloride, ammonium phosphate, potassium nitrate, and calcium nitrate. *Ophiobolus graminis* grew well in all the media containing egg albumen, casein, and peptone, but not in the media containing other nitrogenous sources irrespective of the source of carbon. Weight measurements of the growth of *O. graminis* were made with dextrose, sucrose, maltose, starch, dextrin, and lactose when used in

combination with egg albumen, casein, and peptone. In all cases casein was the poorest source of nitrogen and peptone the best. It is not possible to tell from the data at hand which is the most favorable source of carbon, since *O. graminis* is able to utilize egg albumen, casein, and peptone themselves as a source of carbon. Growth is abundant in the presence of these three proteins when no other source of carbon is present. However, growth is better in all of these when a carbohydrate is present in the medium.

EFFECT OF DIFFERENT HYDROGEN-ION CONCENTRATIONS

Additional experiments were conducted to determine whether the failure of certain compounds to support growth of *Ophiobolus graminis* might not be due to an unfavorable hydrogen-ion concentration. The compounds selected for study were ammonium nitrate, ammonium sulphate, ammonium carbonate, and ammonium chloride. Four batches of media were prepared, each containing a different one of these as a source of nitrogen. Each portion was subdivided into four parts and each part adjusted to a different hydrogen-ion concentration. The range was from pH 4.8 to 8.4. The growth in these different media was in no case greater than the neutral check, which had no nitrogen.

EFFECT OF GROWTH-PROMOTING MATERIALS

Separate batches of media containing the nitrogenous compounds potassium nitrate, ammonium sulphate, ammonium dihydrogen phosphate, ammonium oxalate, and glycine were each subdivided into five parts. A trace of a compound of copper, sodium, silicon, zinc, and aluminum was added separately to each subdivision. In no case did the growth of *Ophiobolus graminis* in these cultures differ from that of the checks containing the several nitrogen compounds without the addition of these elements.

Brenner [3] mentioned that traces of ammonium salts have been found to aid in the assimilation of the alkaloids by *Aspergillus niger* and *Penicillium glaucum*. Traces of ammonium chloride, ammonium tartrate, ammonium formate, and ammonium phosphate were therefore added separately to different samples of media containing caffeine and nicotine. In no case was the growth of *Ophiobolus graminis* improved. Both caffeine and nicotine as a source of nitrogen in Czapek's solution will support certain soil fungi and bacteria, but not *O. graminis*.

Mockeridge [4] did considerable work with accessory factors or growth-promoting substances for green plants. He found that water-soluble material from leafmold, well-rotted manures, fertile manured soils, and well-decayed peat greatly promote the growth of *Lemna major* in nutrient solution. This was not due solely to nutritive action. The writer tried a similar type of experiment with *Ophiobolus graminis*, using a few cubic centimeters of water extracts from garden soil, leafmold, stable manure, chicken manure, and peat to 50 cc of the medium containing the nitrogen compound to be tested. The source of nitrogen in the medium was ammonium chloride. The media were steam sterilized and the organisms were then added. Any

[3] BRENNER, W. DIE STICKSTOFFNAHRUNG DER SCHIMMELPILZE. Centbl. Bakt. [etc.] (II) 40: 555–647. illus. 1914.

[4] MOCKERIDGE, F. A. THE OCCURRENCE AND NATURE OF THE PLANT GROWTH-PROMOTING SUBSTANCES IN VARIOUS ORGANIC MANURIAL COMPOSTS. Biochem. Jour. 14: [432]–450. 1920.

growth that occurred could be accounted for only on the basis of the nitrogen introduced with the water extracts.

Willaman [5] reported that peptone has the power to promote the growth of *Sclerotinia cinerea* in a pure salt and sugar medium. Since *Ophiobolus graminis* can utilize peptone, casein, and egg albumen as sources of nitrogen, they were tested in small amounts as sources of growth-promoting material. They were used in connection with ammonium chloride, sodium nitrate, glycine, uric acid, and gelatin; the control was without nitrogen. In no case was extra growth noted except that which could be accounted for by the nitrogen added in the peptone, casein, or egg albumen.

Czapek's medium having urine as a source of nitrogen supported a good growth of *Ophiobolus graminis*. However, small additions of urine to Czapek's medium containing ammonium chloride did not make the ammonium chloride available. Accordingly, urine does not contain any growth-promoting material for *O. graminis* with respect to ammonium chloride.

NITROGEN PRESENT IN PLANT MATERIALS

Ophiobolus graminis will grow on a large number of different plant decoctions as well as on vegetable tissue. The following is a partial list of decoctions showing the diversified types on which it will grow: Onion, carrot, potato, wheat leaves, sweetpotato, dandelion leaves, radish tops and roots, bluegrass leaves, elm leaves, green beans, iris leaves, and spinach leaves. Approximately 300 g of each of these materials was boiled in 1,000 cc of water to extract the juices, and then dextrose was added at the rate of 20 g per liter. Onion, carrot, and potato tissue are favorable media. Wheat, barley, and oat seed also support good growth. It is probable that the nitrogen consumed in plant decoctions is of a proteinaceous nature.

SUMMARY

Ophiobolus graminis, the parasite causing take-all of wheat, has been found to be specific in its nitrogen food requirements when the nitrogen was supplied in a modified Czapek's nutrient solution. Egg albumen, casein, peptone, and nucleic acid were the only compounds utilized by the fungus. The nitrogen of the other materials tried, both inorganic and organic, was unavailable. This unavailability was not affected by the hydrogen-ion concentration of the media, by the source of carbon, nor by the presence of inorganic or organic growth-promoting materials. Many plant decoctions as well as the tissue of several plants were favorable media for *O. graminis*.

Rhizopus sp. and *Penicillium* sp. were specific in some cases as to the kind of nitrogen compound they could utilize. However, they could utilize many compounds that were unavailable to *Ophiobolus graminis*.

[5] WILLAMAN, J. J. THE FUNCTION OF VITAMINES IN THE METABOLISM OF SCLEROTINIA CINEREA. Jour. Amer. Chem. Soc. 42: 549–585, illus. 1920.

THE INSECTICIDAL ACTION OF ACID LEAD ARSENATE ON THE LARVAE OF THE JAPANESE BEETLE IN DIFFERENT TYPES OF SOIL[1]

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INTRODUCTION

In a previous report from this laboratory (*10*) [2], it was shown that acid lead arsenate is the most dependable of the inorganic arsenicals for killing larvae of the Japanese beetle (*Popillia japonica* Newman) in the soil. The larvae are killed by ingesting a fatal dose of the arsenical as they burrow through the poisoned soil, or by feeding on rootlets growing in the soil. The time required for the material to be effective depends upon the stage of development, the degree of activity, and the susceptibility of the larvae. It is also influenced by the type of soil, for it has frequently been observed in the field that larvae of the Japanese beetle are more readily destroyed by acid lead arsenate in certain types of soils than in others. During the spring of 1935 a study was made of types of soil representative of the area within 100 miles of Moorestown, N. J., to determine whether the insecticidal effectiveness of the material was correlated with any intrinsic property of the soil.

DESCRIPTION OF THE SOILS

The soils used in this study included samples of soils developed from glacial till, soils developed from materials formed in situ by decomposition of underlying rocks, and soils transported from elsewhere and laid down by water. They were taken from various sections of New Jersey (*6, 7, 8, 11, 12, 13, 14, 15, 16, 17*), and can be broadly classified, according to physiographic location, as follows: Soils of the glaciated region, limestone valley soils, Piedmont Plateau soils, and Coastal Plain soils. These groups are further divided into soil series on the basis of structure, color, and drainage characteristics. The 15 types of soils selected for this study are described below.

SOILS OF THE GLACIATED REGION

Dover loam consists of a brown to light-brown, gritty loam, 8 to 15 inches deep, overlying yellowish-brown, gritty clay or gritty clay loam, which passes quickly into yellowish or reddish-yellow, friable sandy clay. It is derived from glacial till. Gravel and occasional boulders of limestone, gneiss, quartzite, and chert are present. Outcrops of limestone occur in many places. The soil is well drained.

Washington loam is a brown, mellow loam, 10 to 15 inches deep, overlying reddish-yellow, moderately friable silty clay, which usually is gritty, containing small fragments of partly decomposed rock.

[1] Received for publication July 3, 1936; issued January, 1937.
[2] Reference is made by number (italic) to Literature Cited, p. 778.

Angular fragments and pebbles of gneiss, quartzite, flint, and occasionally limestone are present. The parent material consists of old glacial drift, in most cases overlying limestone or shale.

Dutchess shale loam is a light-brown to gravish-brown silt loam, 6 to 10 inches deep. The subsoil ranges from a yellowish-brown silt loam to silty clay loam. The depth to bedrock probably does not average more than 2 feet, and shale outcrops in places on tops of hillocks and crests of ridges. The rock fragments in the soil vary from small chips to platy pieces 4 to 5 inches across. Foreign boulders and gravel of quartzite and gneiss and occasionally limestone occur. The soil is well drained; in fact, crops often suffer from lack of moisture during dry periods, especially where the bedrock is close to the surface.

LIMESTONE VALLEY SOILS

Hagerstown silt loam was the only soil obtained from the limestone valley. The soils of this locality have been derived from limestone, and represent the residual material left upon removal in solution of the calcium and magnesium carbonates. The soil is thus composed of impurities in the original rock. The surface soil is a brown, mellow silt loam, 10 to 15 inches deep, overlying slightly reddish yellow, moderately friable silty clay, which shows no important change to a depth of 3 feet or more. Fragments and small outcroppings of limestone are of common occurrence. The topography is gently rolling and the drainage excellent.

PIEDMONT PLATEAU SOILS

Penn shale loam consists of Indian-red silt loam, 8 to 14 inches deep, over brighter Indian-red, brittle clay derived from Triassic formations. There is an abundance of shale fragments from the surface down, and usually the parent shale occurs within the upper 3-foot layer. Often the lower subsoil is friable, owing to the larger quantity of partly decomposed shale material present. Shale fragments range from less than one-eighth to one-half inch or more in thickness and have practically the same color as the soil. This soil occupies gently rolling ridges, hillsides, and stream slopes. Run-off is excessive, and crops often suffer from lack of moisture, especially during dry seasons.

Chester gravelly loam is a light-brown or brown, gritty loam, 8 to 12 inches deep, overlying brownish-yellow or yellow, friable, gritty clay loam or clay. Angular fragments of gneiss are abundant over the surface and through the soil and subsoil, giving the former a decidedly gravelly character. Varying quantities of mica are found. In places the soil is shallow, and the yellowish subsoil comes near enough to the surface to give plowed fields a spotted brownish and yellowish appearance. This soil is derived from the weathered products of coarse-grained gneiss of pre-Cambrian age. Fragments of parent rock ranging in size from huge masses to small gravel are encountered. The soil is well drained, except in a very few places bordering depressions on ridges, where the underdrainage is imperfect.

COASTAL PLAIN SOILS

The soil-forming materials of the Coastal Plain in New Jersey consist of unconsolidated water-laid sands, clay, gravel, greensand, and marly clay. The beds are composed of materials that were trans-

ported from older land areas and deposited, in part, under marine conditions. Since the elevation of this part of the ocean floor, streams, winds, and other agencies of erosion have removed some of the materials and more or less modified and rearranged the original deposits.

Sassafras sand under virgin conditions consists of a layer of gray to brown sand, about 1 inch thick, underlain by a brownish-gray sand, which at a depth of 5 to 8 inches passes into orange or reddish-yellow slightly loamy sand subsoil. The drainage is good to excessive, and in dry seasons crops are likely to suffer greatly from drought.

Sassafras sandy loam consists of a light-brown or brown sandy loam or loamy sand from 6 to 12 inches deep. This rests on orange-yellow or reddish-yellow sandy loam, which at a depth of 20 inches or less passes into yellowish-red, reddish-yellow, or dull-red friable sandy clay, generally becoming lighter in texture at a depth of about 3 feet. The soil is considered one of the most productive for general farm crops. It is easy to work and holds up well during protracted dry spells.

Collington sandy loam consists of brown loamy sand or sandy loam from 8 to 12 inches deep, underlain by yellowish-brown friable sandy clay continuous in most places to a depth of more than 3 feet. The subsoil contains enough greensand marl to give it a decidedly greenish cast. The soil is easy to work and appears to withstand protracted periods of dry weather.

Colts Neck loam is red or brownish-red loam to a depth of 7 or 8 inches. The subsoil is red or reddish-brown friable sandy clay loam or sandy clay. In the lower part of the subsoil, usually below a depth of 30 inches, the texture becomes somewhat lighter, the structure more porous, and the color changes gradually to a yellowish red. The Colts Neck soils are characterized by the presence of ferruginous fragments consisting usually of what are known as "clay ironstones." There is wide variation in the number as well as in the size and shape of these fragments. In the level areas they are few and very small, resembling the fragments of shale in soils derived from shale material. In some spots, however, they may compose as much as 30 to 40 percent of the soil mass, and attain a weight of several hundred pounds. The soil is well drained.

Keyport loam is a grayish-brown loam to a depth of 12 inches. It is underlain by yellow moderately friable clay loam, which, at a depth ranging from 15 to 24 inches, grades into somewhat plastic clay containing gray and yellow spots indicative of incomplete oxidation. Quartz particles may be present on the surface. Both the subsoil and the substratum are comparatively heavy and in wet weather are subject to excessive ground water, but this is soon disposed of because of the comparatively good surface drainage.

Lakewood sand is light-gray or white loose sand ranging from 6 to 24 inches in thickness. In the untilled soil there is in most places a thin layer beneath the forest mold which contains enough organic matter to give the sand a darker color. The subsoil is yellow, coffee-brown, or golden-yellow sand, and in most cases is uniform in character to a great depth. Lakewood sand is easily cultivated, but the crops suffer from drought.

Lakewood sandy loam, deep phase, consists of about 4 to 8 inches of white sand, frequently a little grayish in the surface inch owing to the presence of organic matter, underlain by yellow or orange-yellow loamy sand, passing, at depths ranging from about 12 to 24 inches, into reddish-yellow, orange-yellow, or yellow sandy loam to sandy clay, which, at depths of about 30 inches, passes abruptly into yellow friable sandy clay. This type occupies high, well-drained areas with rolling topography. The drainage is thorough to excessive. Practically none of the soil is cleared. The scrubby forest covering it consists of scrub oak and pine with an undergrowth of bracken and huckleberry.

Elkton silt loam is a grayish silt loam from 5 to 12 inches deep, underlain by bluish-gray or almost white silty loam which, at a depth varying from 15 to 20 inches, grades to light bluish-gray silty clay loam mottled with yellow or rust brown. The surface soil is slightly compact and contains very little organic matter, even in timbered areas. The soil occupies flat or depressed areas and is generally poorly drained. Early in the spring much of it is so covered with water that the areas resemble ponds. These become dry as the season advances.

Woodstown sandy loam consists of a light-brown or brown loamy sand, sandy loam, or heavy sandy loam underlain at a depth of about 6 to 8 inches by a yellowish sandy loam or heavy sandy loam which in places shows a slight reddish cast. At a depth between 20 and 24 inches mottled yellow and light-gray or bluish-gray sandy clay is reached. This becomes more sandy below, and coarse sand and gravel occur at a depth of less than 40 inches. The soil occupies flat, low areas, mainly about stream heads and near areas of tidal wash. Underdrainage is imperfect and water is found in many places within the 3-foot depth.

DETERMINATION OF THE COEFFICIENTS OF EFFECTIVENESS OF ACID LEAD ARSENATE IN DIFFERENT TYPES OF SOIL

Each soil used in this study was obtained from the locality in New Jersey in which it predominated. As far as possible, the samples were taken from pastures, wood lots, and fields that were not under cultivation, as such soils had not been much modified from their natural condition.

In the field differences in texture, moisture content, and vegetation make it difficult to obtain a uniform distribution of the poison. This, together with the wide variation in the density of the larval population, introduces complications which practically preclude any satisfactory comparison of the soils under natural conditions without extensive experimentation. Many of these factors can be controlled in the laboratory. By placing the soils in earthen pots and maintaining them at their best moisture content at a constant temperature, by having the same vegetation growing in each soil, and by introducing the same number of larvae in the same stage of development and from the same source, and leaving them in the soil for the same length of time, as outlined by Fleming and Baker (*10*), it is possible to compare the insecticidal action of the material in the different soils without extensive work in the field.

Each soil was treated with different concentrations of acid lead arsenate, ranging from 250 to 2,000 pounds per acre, to determine the concentrations that would kill 50 percent of the larvae. Two hundred third-instar larvae were used for each dosage. The effectiveness of acid lead arsenate in the different soils was expressed as the coefficient of effectiveness in Sassafras sandy loam, which was used as a standard soil.

The coefficients of effectiveness, arranged in order of decreasing effectiveness, are given in the following tabulation:

Soil type:	*Coefficient of insecticidal effectiveness*
Lakewood sand	2.18±0.56
Sassafras sand	2.02± .36
Woodstown sandy loam	1.14± .19
Lakewood sandy loam	1.12± .16
Dutchess shale loam	1.01± .13
Sassafras sandy loam	1.00± .13
Washington loam	.98± .15
Dover loam	.97± .15
Hagerstown silt loam	.86± .10
Keyport loam	.81± .12
Chester gravelly loam	.69± .10
Elkton silt loam	.69± .10
Penn shale loam	.65± .07
Collington sandy loam	.46± .07
Colts Neck loam	.30± .05

It is apparent that the effectiveness of lead arsenate as a stomach poison varies in the different soils. In a previous report (*10*) it was shown that the arsenates of aluminum, barium, calcium, iron, lead, magnesium, manganese, and zinc were toxic to Japanese beetle larvae. Recently it was found that such lead compounds as the acetate, borate, carbonate, chloride, fluoride, and acid phosphate were not toxic to the larvae, even when applied at the rate of 2,000 pounds per acre. It may be concluded, therefore, that the insecticidal action of lead arsenate on this insect in the soil is due entirely to the arsenate radical and that the variation in the toxicity of the compound in different soils is due to the reaction of the arsenate radical with the colloidal matter or salts in the soil to form other arsenates of varying toxicity.

CORRELATION BETWEEN INSECTICIDAL ACTION OF ACID LEAD ARSENATE AND CERTAIN CONSTITUENTS OF THE SOIL

The total content of certain soil constituents in the various soils (*1, 2, 3, 4, 5*) is given in table 1. A multiple-correlation analysis was made of the data, according to the procedure outlined by Ezekiel (*9*), to determine the extent to which the variation in the insecticidal action was associated with the variation in the total nitrogen, phosphoric acid, potassium, calcium, magnesium, and carbon in the soils. It was found that the correlation between the coefficient of insecticidal effectiveness and the total plant food was insignificant.

TABLE 1.—*Average quantities of certain soil constituents in the surface soil of the types used in the present study*

Soil type	Nitrogen (N)	Phosphoric acid (P_2O_5)	Potassium (K_2O)	Calcium (CaO)	Magnesium (MgO)	Carbon (C)
	P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
Lakewood sand	300	50	340	2,080	970	2,232
Sassafras sand	560	740	3,890	1,410	1,710	3,520
Lakewood sandy loam	150	100	430	350	140	3,940
Dutchess shale loam	1,870	2,610	17,700	3,260	16,080	20,800
Sassafras sandy loam	1,020	1,180	8,050	2,610	4,130	8,980
Washington loam	1,135	1,195	23,405	3,975	7,440	12,500
Dover loam	1,790	1,740	38,160	6,790	11,040	20,070
Hagerstown silt loam	1,360	1,800	58,390	7,440	15,680	14,050
Keyport loam	1,330	970	11,490	1,190	3,040	14,140
Chester gravelly loam	2,135	2,090	23,360	10,560	10,230	22,200
Elkton silt loam	1,190	650	12,190	4,130	2,030	10,300
Penn shale loam	1,800	1,310	21,280	4,060	17,520	17,010
Collington sandy loam	990	3,130	22,940	5,040	10,130	9,390
Colts Neck loam	1,180	9,370	16,010	1,490	3,070	14,070

CORRELATION BETWEEN INSECTICIDAL ACTION OF ACID LEAD ARSENATE AND CONTENT OF WATER-SOLUBLE CONSTITUENTS AND ACIDITY OF THE SOIL

The different soils were analyzed according to the procedure outlined by Spurway (*18*) to determine the quantities of water-soluble soil constituents. The hydrogen-ion concentration was determined by the potentiometer. Negative tests were obtained for water-soluble carbonates, nitrites, sulphates, aluminum, ferrous iron, and sodium. The positive results are given in table 2.

TABLE 2.—*Coefficient of effectiveness, hydrogen-ion concentration, and content of water-soluble constituents of the different types of soil used in the multiple-correlation analysis*

Soil type	Coefficient of effectiveness (X_1)	pH (X_2)	Chlorides (X_3)	Nitrates (X_4)	Phosphates (X_5)	Ammonia (X_6)	Calcium (X_7)	Magnesium (X_8)	Potassium (X_9)	Manganese (X_{10})
			P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
Lakewood sand	2.18±0.56	4.59	0	10	2.50	2	30	5	5	0
Sassafras sand	2.02± .36	4.30	10	20	1.00	10	60	1	5	1
Woodstown sandy loam	1.14± .19	5.73	10	20	.50	1	100	7	5	0
Lakewood sandy loam	1.12± .16	4.61	0	5	1.00	3	0	3	5	0
Dutchess shale loam	1.01± .13	5.53	5	10	.00	10	90	7	5	5
Sassafras sandy loam	1.00± .13	4.96	5	20	.75	1	20	3	0	1
Washington loam	.98± .15	7.46	20	15	.00	2	150	7	10	5
Dover loam	.97± .15	6.08	5	5	.50	2	40	7	20	2
Hagerstown silt loam	.86± .10	7.38	0	20	.50	2	100	7	20	1
Keyport loam	.81± .12	5.05	10	5	.00	7	100	7	20	0
Chester gravelly loam	.69± .10	5.91	20	20	.75	1	100	7	10	1
Elkton silt loam	.69± .10	4.27	15	20	.50	1	0	0	0	0
Penn shale loam	.65± .07	6.03	0	20	.75	2	40	7	5	2
Collington sandy loam	.46± .07	4.61	5	15	.00	5	40	7	20	1
Colts Neck loam	.30± .05	5.45	30	10	.00	3	60	7	20	1

A multiple-correlation analysis was made of the data to determine the extent to which the variation in the effectiveness of acid lead arsenate as an insecticide was associated with the variation in the

hydrogen-ion concentration and with the various water-soluble constituents of the soil. The coefficient of multiple correlation, $\overline{R}$, adjusted for the number of cases, was 0.877; the coefficient of multiple determination, $\overline{R}^2$, was 0.769, and the adjusted standard error of estimate, $\overline{S}$, was 0.244. Since the coefficient of multiple determination showed that about 77 percent of the variance in the insecticidal action was associated with the water-soluble constituents of the soils, it is apparent that the water-soluble constituents of the soil have the greatest influence on the effectiveness of an insecticide.

The net regression coefficients, the beta net regression coefficients, the coefficients of part correlation, and the coefficients of part determination were calculated according to the procedure outlined by Ezekiel (*9*), and are given in table 3. It was found that an increase in water-soluble phosphates, calcium, manganese, ammonia, or potassium increased the effectiveness of acid lead arsenate as an insecticide, and that an increase in magnesium, hydrogen-ion concentration, chlorides, or nitrates decreased its effectiveness.

TABLE 3.—*Net regression coefficients, coefficients of part correlation, coefficients of part determination, and beta net regression coefficients obtained from correlating the coefficients of insecticidal effectiveness with the proportion of water-soluble constituents and the acidity in various types of soil*

Independent variable	Net regression coefficient [1]	Coefficient of part correlation	Coefficient of part determination	Beta net regression coefficient	Percent or weight of the beta coefficients
Phosphates (X_5)	0.676004	0.873	76.1	0.858	28.0
Calcium (X_7)	.008328	.828	68.5	.709	23.1
Magnesium (X_8)	−.104969	.729	53.1	−.511	16.7
Chlorides (X_3)	−.012966	.427	18.3	−.227	7.4
pH (X_2)	−.114845	.426	18.3	−.226	7.4
Manganese (X_{10})	.050973	.356	12.7	.183	6.0
Nitrates (X_4)	−.010381	.256	6.6	−.127	4.2
Ammonia (X_9)	.020212	.249	6.2	.124	4.0
Potassium (X_6)	.006452	.202	4.1	.099	3.2

[1] In terms of the coefficient of insecticidal effectiveness. The value of the constant term for the net regression equation is 1.337608.

The importance of each of these independent variables may be measured by the coefficient of part correlation and by the coefficient of part determination. In table 3 it is seen that the variation in concentration of phosphates in the soil accounted for 76 percent of the variation in insecticidal effectiveness of acid lead arsenate not explained by variation in the concentrations of the other water-soluble constituents; similarly, that calcium accounted for 68 percent, and that magnesium accounted for 53 percent. The variation in the hydrogen-ion concentration or in the concentration of chlorides, manganese, nitrates, ammonia, or potassium was a minor factor in modifying the insecticidal action.

The net regression coefficients show the average change in effectiveness of acid lead arsenate which occurs with each increase of specified units of hydrogen-ion concentration, or in the water-soluble constituents of the soil. The size of the regression coefficients, however, varies not only with the relation between the variables but also with the units by which each variable is expressed. These coefficients can be reduced to another form by stating each of the variables in

units of its own standard deviation. In this form the net regression coefficients are termed "beta" coefficients and the relative importance of each variable can be determined. When the beta coefficients are compared with the coefficients of part correlation, it is seen that in the two sets of measurements the order of importance of the nine variables is the same. The last column of table 3 indicates the percentage or weight of each independent factor in the whole correlation study. It is hardly to be expected, in a problem consisting of nine independent variables, that significant results can be obtained for each variable.

SUMMARY AND CONCLUSIONS

Larvae of the Japanese beetle are killed by ingesting acid lead arsenate while burrowing through poisoned soil or feeding on rootlets growing in the soil. The insecticidal action may be attributed entirely to the arsenate radical, for arsenates of other metals are toxic, and other lead salts that were tested were nontoxic.

In a study of the effectiveness of acid lead arsenate in 15 types of soil from New Jersey, it was found that the coefficient of insecticidal action ranged from 0.30 in Colts Neck loam to 2.18 in Lakewood sand. The difference in the action of acid lead arsenate appeared to be associated with some intrinsic property of the soils. It is probable that the variation in toxicity is due to the reaction of the arsenate radical with the colloidal matter or salts in the soil to form arsenates of varying degree of toxicity.

There was an insignificant correlation between the coefficient of insecticidal effectiveness and the total nitrogen, phosphates, potassium, calcium, magnesium, and carbon in the soils.

The water-soluble constituents of the soils accounted for 77 percent of the variance in the insecticidal action. The concentrations of water-soluble phosphates, calcium, and magnesium appear to be the most important factors influencing the effectiveness of acid lead arsenate. The greater the concentrations of soluble phosphates and calcium in the soil when acid lead arsenate is applied, the more effective it is, and the greater the concentration of soluble magnesium the less effective it is. The influence of soluble chlorides, manganese, nitrates, ammonia, and potassium in the soil on insecticidal action is of minor importance. Although the hydrogen-ion concentration is a minor factor, the nature of the radicals producing the acidity or alkalinity being more important, it is apparent that acid lead arsenate is generally more effective in acid than in alkaline soils.

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INFLUENCE OF BLACK POINT DISEASE, SEED TREATMENT, AND ORIGIN OF SEED ON STAND AND YIELD OF HARD RED SPRING WHEAT [1]

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INTRODUCTION

In 1934 wheat seed carrying the black point disease was planted to determine what effect if any would be produced on the stand and yield of the progeny. In addition, a study was made of the effect of Ceresan as a seed treatment and the effect on subsequent yield and stand of inoculating seed with bunt spores. Yield and stand of plants from large seed grown in the greenhouse were compared with yield and stand of plants from smaller seed grown out of doors, and, finally, a comparison was made of some hybrid selections with one of the parents, Ceres. While the experiment may be somewhat complex, it is not believed to be less accurate because of the several factors considered. Indeed, Fisher and Wishart [2] advocate complex experiments wherever possible because of the very high degree of precision that may be attained in such experiments. In the present experiment the degrees of freedom for the error are 33. Thus the standard error was small in comparison with the mean, as will later appear.

BLACK POINT INFECTION AND SIZE OF KERNEL

The black point carried by the seed resulted from infection during the growing season of 1933. The infections were caused almost entirely by *Helminthosporium sativum* and an *Alternaria*.[3] Because of the serious nature of *Helminthosporium* infection in wheat it would appear undesirable to plant seed obviously infected with the organism. Güssow [4] reported that the seedlings from black point wheat appeared to be less vigorous than those from sound kernels. Henry [5], experimenting with Marquis wheat containing kernels infected with black point, found that germination was injured both in the greenhouse and outside. Two pairs of rod rows were planted by him in the nursery on different dates. The first pair, planted on May 20, showed a yield from the normal seed of 250 percent of that from the diseased seed. With the other pair, planted 5 days later, the difference was trifling. This experiment carried no replications.

Many experiments have been conducted to determine the influence of size of kernel on yields. Percival [6], experimenting with three

[1] Received for publication June 11, 1936; issued January 1937. Paper no. 22 of the Journal series of the North Dakota Agricultural Experiment Station.

[2] FISHER, R. A., and WISHART, J. THE ARRANGEMENT OF FIELD EXPERIMENTS AND THE STATISTICAL REDUCTION OF THE RESULTS. Imp. Bur. Soil Sci., Tech. Comm. 10, 24 pp. London. 1930.

[3] Dr. H. B. Humphrey, of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, identified the organisms.

[4] GÜSSOW, H. T. REPORT OF THE DOMINION BOTANIST. Canada Dept. Agr. Expt. Farms, Ann. Rept. 1910-11:240. 1911.

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varieties of wheat, planted kernels weighing 25 and 50 mg in each of 5 years and found that the larger kernels produced the larger yields. The gains in yield ranged from nearly 40 to 50 percent. In the present experiment the large kernels were grown under greenhouse conditions, and the smaller kernels out of doors. A review of the literature fails to show any comparison of growth and yield in a crop resulting from the planting of wheat kernels of different sizes which had been grown under outside and inside environments.

MATERIAL AND METHODS

The seed consisted of two sorts. One was the Ceres variety of wheat (*Triticum aestivum* L.) and the other was a composite sample of three hybrid selections of the F_6 seed of the cross Ceres × (Hope × Florence). The seed coming from the greenhouse was F_7 seed grown unselected from the F_6 seed. The additional generation would scarcely modify the genotype of the seed. As laboratory tests indicated high germinating capacity for both lots, any difference in results that might be obtained from the two lots could scarcely be due to inferior seed in one of the lots. Certain characters of the two lots are shown in table 1.

TABLE 1.—*Data on the seed lots used*

Variety or selection	Condition	Parent seed grown	Weight per 1,000 kernels	Relative weight of kernels	Seed used per acre
			Grams		*Pounds*
Ceres× (Hope×Florence)	Healthy	Outside	35.5	112	85
Do	Diseased	do	37.5	118	90
Do	Healthy	In greenhouse	49.0	154	117
Ceres	do	Outside	31.8	100	76

Seed of each of the four classes was given three kinds of treatment. One portion was planted as a control, another was treated before planting with New Improved Ceresan, and a third was inoculated with bunt spores. The seed for the first two classes, healthy and diseased, consisted of a single lot, and out of this a sufficient number of healthy and black point-infected kernels were hand picked for the experiment. The kernels classed as healthy bore no outward sign of infection.

The plantings were made in quadruplicate in 8-foot rows, 200 kernels being used for each row, and the rows were randomized. Random planting evidently should compensate for lack of guard rows in this experiment since different rates of seeding were immediately adjacent. Planting was done by hand on April 21, and emergence was in progress by April 30. A stand count was taken on May 5 and a second count on May 15. The first revealed no critical information as to relative quickness of germination.

STAND

The stand counts secured on May 15 in seedlings per 8-foot row are shown in table 2.

TABLE 2.—*Stand counts secured May 15, per 8-foot row, from 200 kernels planted*

Planting no.	Stand for seed type indicated												Average
	Black point-infected			Healthy			Greenhouse-grown			Ceres variety			
	Control	Treated	Bunted	Control	Treated	Bunted	Control	Treated	Bunted	Control	Treated	Bunted	
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
1	159	164	180	168	171	167	198	193	182	166	172	164	174
2	175	165	171	170	173	180	190	198	194	180	173	179	179
3	162	163	168	166	173	176	188	193	192	179	178	169	176
4	171	169	161	168	180	170	191	195	193	170	163	177	176
Percentage of stand	83.4	82.6	85.0	84.0	87.1	86.6	95.9	97.4	95.1	86.9	85.8	86.1	------

The data in table 2 were analyzed according to Fisher's [7] analysis of variance method. The only z value of significance was that for the four classes, and this at 1.98 was of high value, as would be expected from the relatively large stands shown from the greenhouse seed. The standard error was calculated as 5.3, which is 3.01 percent of the mean of 176. The significant difference for a mean of four rows is 8. The number of seedlings from bunt-inoculated seed in the first two classes was greater than for the control but not significantly so. For Ceres the seedlings from bunt-inoculated seed were essentially equal to the number of seedlings from seed treated with Ceresan. The average stands for the seven units are given in table 3. The number of seedlings from bunt-inoculated seed in the first two classes was greater than for the control but not significantly so. For Ceres the seedlings from bunt-inoculated seed were essentially equal to the number of seedlings from seed treated with Ceresan. The average stands for the seven units are given in table 3.

TABLE 3.—*Average stand counts secured May 5 and May 15 per 8-foot row from 200 kernels planted, using seed of kinds indicated*

Description of seed	Stand count on—		Description of seed	Stand count on—	
	May 5	May 15		May 5	May 15
	Number	*Number*		*Number*	*Number*
Black point-infected	139	167	Control	144	175
Healthy	148	172	Treated with Ceresan	145	176
Greenhouse-grown	161	192	Inoculated with bunt spores	153	176
Ceres variety	141	173			

Considering the stand count for the first four classes taken May 15, the value for significant difference is 4.6. Thus the black point seed had a significantly lower stand than the remaining lots. The stand from the greenhouse-grown seed is decidedly larger than for any of the others. Was this increased stand due entirely, or at all, to the larger seed? Other experiments, not reported here, indicate that the

[7] FISHER, R. A., and WISHART, J. See footnote 2.

larger kernel of the seed from the greenhouse was not responsible for the better stand. The weight per 1,000 kernels of the greenhouse-grown seed was 154 percent of that of Ceres, while the weight of the healthy seed was 112 percent. The corresponding averages for the stand count of May 5, also given in table 3, agree fairly well with the count taken on May 15 when all, or nearly all, seedlings had emerged. The stand from the greenhouse-grown seed stands out prominently in the early count, as it does in the later one.

The average date of heading was June 17. Ceres headed nearly 1 day later than did the other wheats, a significant difference.

YIELD OF GRAIN

At harvest the plants were pulled and wrapped separately. Before threshing, counts were made of the number of plants, the number of heads, and the number of bunted heads. The single yields of threshed grain and the average yields in bushels per acre are given in table 4; the analysis of variance is shown in table 5.

TABLE 4.—*Yield of grain per acre secured from various types of seed*

Planting no.	Yield for seed type indicated												
	Black point-infected			Healthy			Greenhouse-grown			Ceres variety			Average
	Control	Treated	Bunted	Control	Treated	Bunted	Control	Treated	Bunted	Control	Treated	Bunted	
	Bu.	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*	*Bu.*
1	41.6	37.2	35.8	32.4	40.2	33.6	36.4	40.0	36.6	42.2	35.4	17.8	35.8
2	38.2	34.8	31.8	37.8	40.4	36.4	44.8	46.4	36.8	36.6	39.8	21.2	37.0
3	39.8	31.6	26.2	34.6	34.6	28.0	41.2	41.8	34.6	28.4	35.6	14.0	32.6
4	34.2	33.0	33.2	32.4	37.0	29.4	37.2	36.8	34.2	36.0	36.4	18.2	33.2
Average	38.5	34.2	31.8	34.3	38.1	31.9	39.9	41.3	35.6	35.8	36.8	17.8	34.6

TABLE 5.—*Analysis of variance for yield*

Due to—	Degrees of freedom	Sum of squares	Mean square	½ $\log_e$	*z* value
Replication	[1] 3	[1] 4,154	1,385		
Experimental conditions	[1] 11	[1] 40,442	3,677	1.8023	
Classes	3	11,546	3,849	1.8242	1.5139
Treatment	2	17,537	8,768	2.2368	1.9265
Interaction	6	11,359	1,893	1.4704	1.1601
Error	[1] 33	[1] 6,138	186	.3103	
Total	47	50,734			

[1] Items comprising total.

The standard error equals 2.73 bushels, which is 7.89 percent of the mean yield of 34.6 bushels. The differences in treatment and in classes are markedly significant as the *z* values indicate. The significant difference of a mean yield of 4 equals 4.1 bushels. The 12 yields in order are shown in table 6, as are also the averages for classes and treatments.

TABLE 6.—*Yields per acre as related to kind of seed used and treatment*

YIELDS TABULATED IN DESCENDING ORDER

Seed	Treatment	Yield	Seed	Treatment	Yield
		Bushels			*Bushels*
Greenhouse-grown	Ceresan	41.3	Healthy	Control	34.3
Do	Control	39.9	Black point-infected	Ceresan	34.2
Black point-infected	do	38.5	Healthy	Bunt-inoculated	31.9
Healthy	Ceresan	38.1	Black point-infected	do	31.8
Ceres variety	do	36.8	Ceres variety	do	17.8
Do	Control	35.8			
Greenhouse-grown	Bunt-inoculated	35.6	Average		34.6

AVERAGE YIELDS

Greenhouse-grown		38.9	Ceres variety		30.1
Black point-infected		34.8		Ceresan	37.6
Healthy		34.7		Control	37.1
				Bunt-inoculated	29.2

Bunt treatment enters into the yields of each of four classes listed in column 1 of table 6, but only Ceres is markedly affected. The significant difference for classes is 2.4 bushels. Thus it is evident that we have three groups significantly different from each other.

If the bunted rows are excluded from the calculations the efficiency of the results is lessened but comparisons are still worth while. The analysis of variance calculated with the 16 bunted rows omitted is given in table 7.

TABLE 7.—*Analysis of variance of yields with bunt-infected rows omitted*

Due to	Degrees of freedom	Sum of squares	Mean square	½ $\log_e$	z value
Replications	[1] 3	[1] 2,558	853		
Experimental conditions	[1] 7	[1] 4,567	652	0.9373	0.5287
Treatment	1	41	41		
Classes	3	2,797	932	1.1163	.7077
Interaction	3	1,729	576	.8757	.3672
Error	[1] 21	[1] 4,754	226	.4086	
Total	31	11,879			

[1] Items comprising total.

The average yield of the rows in which there was no bunt is 37.3 bushels per acre, and the standard error of a single yield is 3.01 bushels or 8.07 percent of the mean yield. The only z value which is significant and which is of interest in these comparisons is that for classes. Average yields now for the various class units are as follows:

	Bushels
Greenhouse-grown	40.6
Ceres variety	36.3
Black point-infected	36.3
Healthy	36.2
Average	37.3

The significant difference for this group is 3.2 bushels, that for the group from greenhouse-grown seed. The remaining yields are nearly identical. So far as could be judged by appearance, the greenhouse seed differed from the healthy and Ceres seed only in size and greater

plumpness. It would seem that if seed size had been the only factor involved in the yield increase there would have been some disparity in yield between Ceres and the other two groups due to difference in seed weight. But the yields from Ceres and the hybrids are essentially the same.

Other data should indicate what characters of the plant contributed to the high yield of the wheat from greenhouse seed. Such data are given in table 8, which shows the percentage of the seedling stand

TABLE 8.—*Stand maturing, mature plants, stooling, and grain yield per plant for various types of seed planted* [1]

Seed	Stand maturing	Mature plants per 200 seeds	Stools per plant	Grain per plant
	Percent	*Number*	*Number*	*Grams*
Greenhouse-grown	92.8	179	1.8	1.13
Healthy	93.5	160	1.8	1.13
Black point-infected	93.1	155	1.9	1.17
Ceres variety	94.1	163	1.7	1.12

[1] Bunted plants omitted.

that matured, the number of mature plants, the degree of stooling, and the weight of grain per plant. The bunted plants are omitted for a more valid comparison.

As compared with the plants from the greenhouse-grown seed the plants from the healthy seed showed no compensation for reduced stand by increased stooling or more grain per plant. Consequently the greenhouse-grown seed showed the same percentage excess in yield of grain that it had in respect to stand. If there had been a compensation in both instances one would expect the stooling from the healthy seed to be larger than that from the greenhouse seed because of fewer plants, and the grain per plant to be greater from the greenhouse seed because of the greater vigor of the plants. Evidently such was not the case.

It is evident from the foregoing data that it was the larger initial stand that provided the greater yield of that portion of the crop grown from the greenhouse seed.

While Ceres showed decidedly greater susceptibility to bunt than did the other wheats, the yields of the Ceres × (Hope × Florence) selections evidently were also affected. The percentage of mature plants secured from the stand of May 15 from the clean and from the bunt-inoculated seed, the grain per plant, and the yields are shown in table 9.

TABLE 9.—*Percentages of mature plants from stand of May 15, weight of grain per plant, and associated yields, for clean and bunt-inoculated seed of various types*

Seed	Mature plants from stand		Grain per plant		Yields per acre		
	Clean	Bunted	Clean	Bunted	Clean	Bunted	Decrease
	Percent	*Percent*	*Grams*	*Grams*	*Bushels*	*Bushels*	*Bushels*
Greenhouse-grown	92.8	89.5	1.13	1.05	40.6	35.6	5.0
Healthy	93.5	92.0	1.13	1.00	36.2	31.9	4.3
Black point-infected	93.1	92.5	1.17	1.01	36.4	31.8	4.6
Ceres variety	94.1	78.0	1.12	.66	36.3	17.8	18.5

The loss of plants from the seedling stage to maturity among the bunted rows is not significant except for Ceres. The differences in amount of grain per plant are significant except from the greenhouse seed. The differences in yield are significant in all cases. It is evident from table 9 that the loss among the plants from black point-infected seed from May 15 on was no greater than that among the plants from healthy seed. The loss of stand among the plants from seed treated with bunt was inappreciable except for the Ceres variety. In that case the loss was severe.

COMPARISON OF SEED TREATMENTS

The effect of treatment with Ceresan and inoculation with bunt spores on stand and other characters is shown in table 10.

TABLE 10.—*Effect of Ceresan treatment and inoculation with bunt spores on stand, number of mature plants produced, stooling, and yield*

Seed treatment	Stand maturing	Mature plants per 200 seeds	Stools per plant	Grain per plant
	Percent	*Number*	*Number*	*Grams*
Control	93.9	163	1.8	1.14
Ceresan	92.8	166	1.8	1.13
Bunt-inoculated	88.0	155	1.8	.93

Treatment with Ceresan evidently had no appreciable effect on the characters shown; inoculating the seed with bunt spores had a harmful effect on both stand and yield.

DISCUSSION

The work here reported indicates that environmental conditions may have a marked influence upon the ability of the organisms carried in black point-infected seed to enter the seedling. The seed used became infected under conditions of high temperature in 1933. Temperatures were also high in 1934 under drought conditions during the development of the plant, but there was little black-point infection. Perhaps such conditions are adverse to the propagation of the disease from the seed. It would be of value to learn under what environmental conditions one might expect severe infestation and injury to result from black-point seed grown under field conditions.

Not much can yet be said as to the differences in stand from seed grown in the greenhouse and seed grown out of doors. The two lots of seed, healthy and greenhouse-grown, both had a germination of approximately 100 percent in the laboratory. It is possible that despite the good laboratory germination certain diseases carried by the seed had a lethal effect upon the seedling only when the seed germinated in soil. It would be a simple matter to test this point. Or perhaps the greenhouse-grown kernel had a better balanced reserve food supply which prevented the loss of the seedling under the stress of germination in the soil.

SUMMARY

As compared with crops from healthy seed, a crop raised from seed infected with black-point disease was not appreciably affected in yield or other characters, except for a slight difference in stand of seedlings.

The application of Ceresan to the diseased seed before planting had no apparent effect upon yield or other characters. As a matter of fact, the black point-infected seed treated with Ceresan yielded 4.3 bushels less than the untreated lot, which is a barely significant difference, but as healthy seed treated with Ceresan yielded 3.8 bushels more than the untreated seed, probably both of these deviations are accidental.

Application of bunt spores to the seed, reduced the yield in all cases to a significant degree, and the loss in yield of Ceres, a susceptible variety, was pronounced.

Yields of the composite hybrid selections from a Ceres × (Hope × Florence) cross were equal to those of the Ceres parent.

The seed grown in the greenhouse, which was exceptionally heavy in kernel weight, produced decidedly larger yields than the other seed tested. The larger yields from the greenhouse-grown seed were evidently due to larger initial and mature stands.

The interlocking type of experiment described in this paper was effective in reducing the standard error because of a relatively large number of degrees of freedom, comparatively small experimental differences thus showing significance.

THE INFLUENCE OF COMMERCIAL FERTILIZERS, POTASSIUM IODIDE, AND SOIL ACIDITY ON THE IODINE CONTENT OF CERTAIN VEGETABLES[1]

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INTRODUCTION

Studies were begun at the Pennsylvania State College in 1931 to ascertain the influence of long-continued fertilizer treatments of field plots on the iodine content of vegetables grown on them, and to determine the extent to which the iodine content of certain vegetables was increased by an application of potassium iodide to field plots on which the acidity had been modified with sulphur or calcium hydroxide.

WORK OF OTHER INVESTIGATORS

Various aspects of iodine fertilization have been studied by other investigators, both with respect to the growth of plants and the yield of crops, and to the iodine content of the plants to which the iodine compounds were applied. A few studies also have been made to determine the influence of the iodine contained in certain fertilizers on the iodine content of vegetables; these studies, however, have dealt with applications of iodine-containing commercial fertilizers to the current crop, and not with long-continued use of these fertilizers on the same plots.

A number of investigators have reported that iodine compounds, either in nutrient solutions, pot cultures, or field plots, have improved the growth and yields of plants, under the conditions stated. Doerell (*8*)[3] found that 3.2 or 4.2 kg of iodine per hectare increased the yield of hops. He suggested that the iodine in commercial sodium nitrate or superphosphate, amounting to about 100 g per hectare, was beneficial to crops. Loew (*22*) reported increases in yield of both tops and roots of radishes fertilized with 0.5 and 0.05 g of potassium iodide to the plot of 20 m^2 as compared with unfertilized checks, the smaller application producing the largest yield. Malhotra (*26*) stated that moderate fertilization of soil with iodine—5.0 mg per kg of soil naturally containing 0.85 mg per kg—stimulated the growth of carrots somewhat. Mazé (*28*) found that to obtain full growth of maize in nutrient solutions of N, P, K, Ca, Mg, S, Fe, Mn, Zn, Si, and Ce from commercial chemical compounds in distilled water, it was necessary to add iodine, boron, aluminum, and fluorine. Scharrer and Schropp (*32*) observed a slight stimulation of growth of wheat growing in gravelly soil with nutrient solutions, when low concentrations of iodine compounds were used. Stoklasa (*35*, *37*)

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[3] Reference is made by number (italic) to Literature Cited, p. 798.

improved the growth of sugar beets with 0.02 g of iodine as potassium iodide in 12 kg of soil per pot culture. Weiske (*40*) found that barley and spinach were stimulated by iodine compounds applied at about 5 to 10 times the rate contained in Chile saltpeter applications. Suzuki (*38*), and Suzuki and Aso (*39*) found that peas, oats, and radishes were stimulated by KI treatment.

Other investigators, however, have reported crop reduction or injury to plants as a result of iodine treatment. Beaumont and Karns (*2*) found a slight decrease in yield of turnips resulting from the application of KI at the rate of 2 kg per hectare. Cotton (*5*) observed injury to buckwheat from iodine as KI in nutrient solutions, in concentrations from 0.13 to 126.9 parts per million. Dafert and Brichta (*6*) found no differences in the growth of barley, mustard, and turnips resulting from the use of iodine in amounts equal to those present in the usual field applications of Chilean nitrate of soda. Doerell (*8*) noted a reduction in yield of hops from applications of 5.4 kg of KI per acre, though the difference was probably not significant.

Engels (*9*) reported no stimulative effect of either KI or $NaIO_3$ on sugar beets and mangels in field plots. Fellenberg (*13*) noted no effect on yield of mangels from 2.0 kg of KI per hectare. Hiltner and Bergold (*21*) reported similar results with 400 to 600 g of KI per acre, with sugar beets. Malhotra (*26*) found that the application of 32.0 mg of KI per kg of soil soon killed carrots growing on it. Scharrer and Schropp (31) observed injury to oats, wheat, rye, and barley, in decreasing order of damage, from the use of iodine salts and noted less unfavorable effects on clay soil than on muck soil. They reported the following order of damage for the different iodine salts: iodide > iodate > periodate. Weiske (*40*) showed that the amounts of iodine compounds present in commercial Chile saltpeter did not influence crops, though 5 to 10 times these amounts were usually injurious, except to barley and spinach. Wrangell (*41*) could find no differences in yield of potatoes, beets, oats, spinach, lettuce, pumpkins, red clover, sprouts, beans, or alfalfa with either 1.5 or 6.0 kg of iodine per hectare in KI or soot, in pot cultures or field plots. Wynd (*42*) found that iodine concentrations from 1 to 20 parts per million from KI, in Shive's nutrient solution, in all cases reduced the growth of tomato plants, and in concentrations above 5 parts per million caused injury to the leaves.

Many of the investigators named above and others have reported increases in the iodine content of plants to which iodine applications had been made. Beaumont and Karns (*2*), Conner (*4*), Densch, Steinfatt, and Günther (*7*), Fellenberg (*10*, *13*, *14*), Hercus and others (*17*, *19*), Hiltner (*20*), Hiltner and Bergold (*21*), Malhotra (*26*), Maurer, Schropp, and Ducrue (*27*), Orr, Kelly, and Stuart (*29*), Pfeiffer and Courth (*30*), Scharrer and Schwaibold (*33*), Scharrer and Strobel (*34*), and Stoklasa (*36*, *37*) all reported increases, in some instances tenfold, in the iodine content of plants receiving iodine applications as compared with that of control plants. The largest amount of iodine found in any plant tissues as the result of fertilization with iodine compounds was 1,419,000 and 1,184,000 parts per billion of dry weight of radish leaves and roots, respectively, reported by Pfeiffer and Courth (*30*). Only Wrangell (*41*) found no increase in iodine content of plants as a result of iodine fertilization.

The iodine contained in commercial fertilizers has been reported by several investigators as increasing the iodine content of the crop, while others have observed no such effect. Conner (*4*) stated that fish meal as a source of nitrogen increased the iodine content of vegetables. Hercus and others (*18, 19*) found that commercial fertilizers increased the iodine content of grasses, but ascribed this effect to improved growth rather than to the iodine content of the fertilizers. Analyses by Fellenberg (*10, 11*), McHargue, Roy, and Pelphrey (*23*), Hercus, Benson, and Carter (*18*), and Hercus and Roberts (*19*) showed that commercial fertilizers of mineral origin contain considerable quantities of iodine. With the exceptions noted above, however, the investigators did not carry out experiments to ascertain whether the iodine contained in the fertilizers actually increased the amount of iodine present in the crop as harvested.

The effect of acidity of the culture medium upon iodine absorption by plants was investigated to some extent by Fellenberg (*15*) and by Malhotra (*26*). The former studied iodine absorption on two very acid soils (pH 4.4 and 4.5) and on two moderately alkaline soils (pH 7.2 and 7.3). He found that carrots obtained more iodine, which was added as potassium iodine, from acid soils in pot cultures than from alkaline soils under similar conditions. He found, further, that acid soils liberated more iodine by catalytic action than did alkaline soils, and that the organic matter of the soil was active in fixing iodine in soils. Malhotra (*26*) found that iodine absorption by carrots in nutrient cultures was lowest at pH 9.0, next lowest at pH 4.0, and highest at pH 6.0; at pH 5.0, 7.0, and 8.0, absorption was intermediate. Densch, Steinfatt, and Günther (*7*) found that liming increased the iodine content considerably when no iodine was added to the soil, but had little effect with the maximum amount of added iodine.

METHODS

The analytical procedure in most of these studies was similar to that described by Frear (*16*), except for certain modifications in the combustion apparatus. According to this procedure, dried, ground samples of plant material are burned with commercial oxygen in a closed apparatus provided with chambers for precipitating smoke and for scrubbing volatile products of combustion; the ashes, precipitated smoke, and scrubbing solutions are collected, evaporated to dryness in excess of alkali, and the iodine compounds in them are extracted with alcohol. After the alcohol has been removed by evaporation, the iodine compounds are dissolved in water, oxidized, and estimated titrimetrically.

The final form of the combustion flask used in these studies was a wide-mouthed (53 mm) 1-liter pyrex Erlenmeyer flask, fitted with a rubber stopper through which were inserted an oxygen inlet tube of 5-mm bore, an outlet tube of 7-mm bore for gaseous products of combustion, and a thick-walled tube of 27-mm bore through which the sample was introduced for combustion, all of pyrex glass. The large tube was about 30 cm in length, and was inserted through the stopper so as to extend well into the body of the flask. The sample of dried, ground vegetable material was introduced through this tube in the way described by Frear. The oxygen inlet tube extended

to the end of the large tube inside the flask, and was bent toward the large tube at that point, so as to deliver oxygen directly to the point at which combustion took place (fig. 1).

The flask was supported by means of clamps in a horizontal position, so that ashes fell from the end of the sample as it was burned; the ashes were not subjected for any considerable length of time to the heat of the flame, as they were when the sample was burned in the vertical position employed by Frear.

The absorption apparatus included, in order, a water-jacketed condenser, a trap vessel for condensed liquids, and three Cottrell precipitators alternated with absorption towers containing glass beads moistened with saturated potassium carbonate solution.

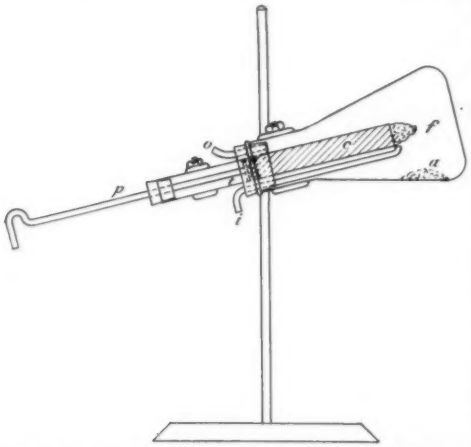

FIGURE 1.—Combustion apparatus for iodine analysis: *p*, Plunger for moving the cartridge of dried, ground sample, *c*, through the tube, *t*; *i*, oxygen inlet; and *o*, outlet for gaseous combustion products; combustion is carried on inside the flask, *f*; and ash, *a*, accumulates as it falls from the burning tip of the cartridge.

Samples were prepared for analysis by slicing, drying at 70° C., grinding in a Wiley mill, and storing in glass-stoppered bottles. Before being ignited they were mixed with about 5 percent of their weight of powdered $CaCO_3$, which was found to improve the smoothness of burning of the sample. In other respects, the analytical procedure was similar to that followed by Frear (*16*), to whose paper the reader is referred for details of the method.

The accuracy of the analytical method was determined by analysis of three different lots of vegetable material before and after the addition of known quantities of potassium iodide. Two lots of tomatoes and one of asparagus were used. Recoveries were made of from 95 to 99 percent of the quantities added. In asparagus, which was found to contain 108 micrograms of iodine, and to which 85 micrograms were added, the total amount recovered was 189 micrograms. With one lot of tomatoes, 378 micrograms of iodine were found after 360 micrograms had been added to the 20 micrograms originally determined in the sample itself; in the second lot, 358 micrograms were found after 360 micrograms has been added to a sample estimated to contain 15 micrograms of iodine.

Analyses were duplicated; if the results of the duplicates were not very closely in agreement, three or more analysis were made.

Tomatoes (*Lycopersicum esculentum* Mill.) sweet corn (*Zea saccharata* Sturt.), and potatoes (*Solanum tuberosum* L.) grown on certain of the plots in a fertilizer experiment with truck crops were analyzed, in an effort to find the relation between iodine content and the amount and kind of fertilizer used. The soil acidity of each plot

was measured to ascertain the relation, if any, between iodine content and soil acidity.

The fertilizers, which varied in the proportions of nitrate of soda, superphosphate, and muriate of potash, were similar in analysis to those that had been applied annually with a few exceptions, since 1917, to the plots from which the samples were taken. (A full description of the fertilizer experiment of which these plots are a part is contained in Pennsylvania Bulletin 210 (*25*). The fertilizers were applied broadcast and were harrowed in before the crops were planted. The particular lots used in the season in which samples were taken (1931) were Chilean nitrate of soda, 16 percent superphosphate, and muriate of potash. The amounts of these materials which were applied to the various plots are given in table 1.

Soil acidity in terms of pH was measured electrometrically, a potentiometer with a quinhydrone electrode being used.

TABLE 1.—*Iodine content of truck crops as influenced by fertilizer treatment and soil acidity*

POTATOES

Fertilizer treatment (pounds per acre)	Plot no.	pH of soil	Yield per acre	Iodine content on basis of—	
				Fresh weight	Dry weight
			Tons	*Parts per billion*	*Parts per billion*
625 superphosphate, 167 muriate of potash	1–8	6.60	4.4	46	180
	4–10	6.94	3.9	48	210
Average			4.2	47	195
600 nitrate of soda, 625 superphosphate, 167 muriate of potash	5–14	6.30	2.8	55	220
400 nitrate of soda, 167 muriate of potash	1–7	6.51	2.7	60	240
	4–11	6.42	3.9	50	200
Average			3.3	55	220
400 nitrate of soda, 938 superphosphate, 167 muriate of potash	5–10	6.80	5.7	41	190
400 nitrate of soda, 625 superphosphate	1–6	6.55	4.3	51	200
	4–12	6.66	2.9	40	150
Average			3.6	45.5	175
400 nitrate of soda, 625 superphosphate, 250 muriate of potash	2–12	6.55	4.5	93	410
	5–6	6.88	4.6	50	210
Average			4.6	71.5	310
Average				53.4	221

TOMATOES

625 superphosphate, 167 muriate of potash	1–8	6.60	13.4	16	230
	4–10	6.73	10.2	8	240
Average			11.8	12	235
600 nitrate of soda, 625 superphosphate, 167 muriate of potash	5–14	6.30	14.7	8	170
400 nitrate of soda, 167 muriate of potash	1–7	7.36	11.1	19	250
	4–11	6.77	8.1	12	220
Average			9.6	15.5	235
400 nitrate of soda, 938 superphosphate, 167 muriate of potash	5–10	6.59	13.2	7	150

TABLE 1.—*Iodine content of truck crops as influence by fertilizerd treatment and soil acidity*—Continued

TOMATOES—Continued

Fertilizer treatment (pounds per acre)	Plot no.	pH of soil	Yield per acre	Iodine content on basis of—	
				Fresh weight	Dry weight
			Tons	*Parts per billion*	*Parts per billion*
400 nitrate of soda, 625 superphosphate	1–6	7.52	17.1	14	300
	4–12	6.87	13.8	17	370
Average			15.5	15.5	335
400 nitrate of soda, 625 superphosphate, 250 muriate of potash	2–12	6.28	17.5	8	160
	5–6	6.38	15.8	13	320
Average			16.7	10.5	240
Average				12.2	241

SWEET CORN

625 superphosphate, 167 muriate of potash	1–8	6.57	2.7	24	90
	4–10	5.96	3.9	25	100
Average			3.3	24.5	95
600 nitrate of soda, 625 superphosphate, 167 muriate of potash	5–14	6.67	3.8	73	250
400 nitrate of soda, 167 muriate of potash	1–7	6.59	2.4	37	140
	4–11	6.09	2.4	24	120
Average			2.4	30.5	130
400 nitrate of soda, 938 superphosphate, 167 muriate of potash	5–10	6.74	4.8	50	190
400 nitrate of soda, 625 superphosphate	1–5	5.85	3.1	33	120
	4–12	6.68	3.8	23	90
Average			3.5	28	105
400 nitrate of soda, 625 superphosphate, 250 muriate of potash	2–12	6.73	3.2	30	100
	5–6	6.43	4.7	33	120
Average			4.0	31.5	110
Average				35.2	132

The plots are listed in table 1 in such a way as to show the effect of the presence or absence of each of the critical plant-food elements, nitrogen, phosphorus, and potassium. The first two plots in the table, plots 1–8 and 4–10, received phosphorus and potassium but no nitrogen; the third plot, 5–14, received the same amounts of phosphorus and potassium as the first two plots, together with a fairly heavy application of nitrogen. The fourth and fifth plots, 1–7 and 4–11, received nitrogen and potassium, but no phosphorus; the sixth plot, 5–10, received the same amounts of nitrogen and potassium as plots 1–7 and 4–11, together with a relatively heavy application of phosphorus. Similarly, plots 1–6 and 4–12 received nitrogen and phosphorus, without potassium, and plots 2–12 and 5–6 received the same amounts of nitrogen and phosphorus, together with a heavy application of potassium. The pH of most of the plots varied but little; the maximum variation for any crop was between 6.28 and 7.52, for tomatoes.

INFLUENCE OF FERTILIZER TREATMENT AND SOIL ACIDITY ON THE IODINE CONTENT OF POTATOES, TOMATOES, AND SWEET CORN

The variation in iodine content of potatoes from the different plots (table 1) was not great, with the exception of those from plot 2–12, which received a generous application of potassium. Potatoes from the second plot receiving the same fertilizer treatment (plot 5–6) did not differ widely in iodine content from those from the other plots. Obviously, there is no relation between either the fertilizer treatment, the acidity of the soil, or the size of the crop, and the iodine content of potatoes grown on the experimental plots. The same is true for tomatoes, although the iodine content of this crop was somewhat more variable among the different plots than was the case with potatoes. The iodine content of sweet corn apparently was increased by nitrate of soda on plot 5–14, as compared with plots 1–8 and 4–10; all other plots, each of which received two-thirds as much nitrate of soda as did plot 5–14, differed but little with respect to the iodine content of the sweet corn grown on them, from the plots without nitrogenous fertilizers.

The average iodine content of sweet corn from all plots, 132 parts per billion of the dry samples, was considerably below that of the tomatoes, 241 parts per billion, and that of the potatoes, 221 parts per billion. The difference between the tomatoes and potatoes in this respect is not significant.

INFLUENCE OF IODINE FERTILIZATION ON THE IODINE CONTENT AND YIELD OF BEANS AND TURNIPS

Studies were carried out in 1931 with beans (*Phaseolus vulgaris* L.) and turnips (*Brassica rapa* L.) to ascertain whether any considerable increase in iodine content might be effected economically by applications of an iodine compound to field plots. Soil acidity was introduced as an experimental variable, by modifying the acidity through additions of hydrated lime or sulphur.

Five plots, each 20 by 24 feet, were marked out on July 20, and materials were applied as follows:

Plot 1—44.0 pounds of hydrated lime (2 tons per acre).
Plot 2—22.0 pounds of hydrated lime (1 ton per acre).
Plot 3—Check.
Plot 4—66.0 pounds of sulphur (3 tons per acre).
Plot 5—110.0 pounds of sulphur (5 tons per acre).

These materials were broadcast and harrowed into the soil. Five rows each of Sure Crop Stringless Wax beans and Purple Top White Globe turnips were seeded on the same day, the beans in rows 30 inches apart, and the turnips in rows 18 inches apart, parallel to the longer side of the plots.

The hydrogen-ion concentration was measured electrometrically from time to time, to ascertain the progress of the change of acidity brought about by the materials applied. The results of these measurements are shown in table 2. The effects of the hydrated lime were immediate, while those of sulphur were not apparent before the second measurement, more than 2 weeks after the sulphur was applied.

TABLE 2.—*pH of plots on four dates during the iodine fertilization experiment*

Plot no.	pH of plots			
	July 27	Aug. 6	Aug. 18	Sept. 22
1	8.33	8.22	8.34	8.23
2	8.25	7.65	8.33	8.16
3	7.73	7.56	7.61	7.60
4	7.74	6.80	6.12	5.85
5	7.73	6.50	5.92	4.95

On August 21 the corresponding half of each plot, including half of each row of turnips and of beans, was fertilized with 12.984 g of potassium iodide, or at the rate of 2.356 kg to the acre. This was distributed by dissolving it in water and sprinkling it evenly upon the surface of the soil, between the rows of beans and turnips, the former of which were beginning to blossom by this time. After the solution had been distributed, the plants were sprinkled thoroughly with water, so that none of the solution would remain on the portions of the plants above ground. The iodine added to the upper 6 inches of soil in this application amounted to approximately 2.0 mg, or 2,000 micrograms, per kilogram, which is about one-half of the total iodine content of certain agricultural soils, as reported by Chatin (*3*), Fellenberg (*12*), Andrew (*1*), and McHargue, Young, and Roy (*24*). Each square foot of soil surface received 0.0413 g of iodine. The application was delayed until this time in order that marked differences in soil acidity might be brought about before the iodine was applied.

The effects of soil acidity and of iodine became evident within 2 weeks after the potassium iodide was applied. Both the beans and the turnips were noticeably more vigorous on the more alkaline lots, and were much less vigorous on the more acid plots. The turnips were especially low in vigor on the plots to which sulphur had been added. Probably this was due, in part, to the unfavorable soil acidity, but to a considerable extent it was attributable to aphid infestation. These infestations were severe only on the plots that had received sulphur applications. The effects of the potassium iodide on the two crops were opposite in character: the turnips were noticeably improved in growth, while the beans were injured, as was shown by the dropping of the lower leaves and the yellowing of the remaining ones, and a general stunting of growth.

The crops were harvested when they reached commercial maturity and their fresh weights recorded. The weights of the green bean pods also were recorded, and the entire harvest from each plot was prepared for iodine analysis. The turnips were weighed and 5-kg samples, made up of entire plants, were taken for analysis. In harvesting and sampling for analysis, a 2-foot strip between the plots, and between the iodine fertilized and unfertilized halves, was excluded. The harvested portions of the half plots, therefore, were 10 by 20 feet, of which 10 by 12.5 feet were occupied by beans, and 10 by 7.5 feet by turnips.

The yields and the iodine content in parts per billion of dry and of fresh weight of the crops from each plot are recorded in table 3. In this table, the halves of the plots to which potassium iodide was applied are designated by the letter A, while the unfertilized halves are designated by the letter B.

TABLE 3.—*Effect of soil acidity and potassium iodide fertilization with KI on the yield and iodine content of beans and turnips*

Plot no.[1]	pH of soil		Beans			Turnips		
	When KI was applied	When crop was harvested	Yield per plot	Iodine content on basis of—		Yield per plot	Iodine content on basis of—	
				Fresh weight	Dry weight		Fresh weight	Dry weight
			Kilograms	*Parts per billion*	*Parts per billion*	*Kilograms*	*Parts per billion*	*Parts per billion*
1A	8.34	8.23	6.75	73	730	37.55	9,496	94,960
1B	8.34	8.23	8.20	26	280	32.12	79	740
2A	8.33	8.16	3.85	84	1,170	27.13	5,692	51,740
2B	8.33	8.16	6.23	57	510	25.99	85	850
3A	7.61	7.60	2.33	71	750	22.50	2,044	19,540
3B	7.61	7.60	4.68	26	260	21.64	180	1,590
4A	6.12	5.85	2.43	56	570	9.89	2,607	24,140
4B	6.12	5.85	3.58	45	490	7.71	209	2,080
5A	5.92	4.95	1.21	122	1,410	7.63	2,148	21,140
5B	5.92	4.95	4.24	54	630	7.03	191	1,910

[1] The halves of plots to which KI was added are designated by the letter A, the unfertilized halves by B.

Table 3 shows that the yield of beans was reduced in every case by the addition of potassium iodide to the soil; the yield of turnips, on the other hand, was increased by iodine fertilization in every case. In general, the yields of both beans and turnips decreased rapidly as the acidity increased.

In turnips, which were stimulated in growth by fertilization with potassium iodide, the iodine content was enormously increased by this means. The increase ranged from a little more than tenfold on plots 3, 4, and 5, to about 120-fold on plot 1, on the basis of fresh weights. On the other hand, the iodine content of the beans, which were injured by potassium iodide, was increased to less than three-fold in all cases. The maximum increases in iodine content of turnips occurred on the most alkaline plots; the increases in iodine content of the beans evidently were not related to the acidity of the soil. On plots treated with potassium iodide, the iodine content of turnips was greater the higher the pH of the soil, except on plot 3; on plots without potassium iodide applications, on the other hand, the iodine content was greater the lower the pH, except on plot 5.

Turnips obtained far greater quantities of iodine from the soil to which no iodine was added than did beans. The fact that the entire plants of turnips were analyzed, however, while only the green pods were used for the measurements of iodine content of beans, should be considered.

On the basis of the yield and iodine content of turnips on plot 1A, the recovery of the added iodine by the turnips on this plot was 0.3536 g, or 11.4 percent of the amount added.

In view of the small recovery of added iodine, the plots were planted in 1932 in the same way as in 1931, but without applying any additional sulphur, hydrated lime, or potassium iodide, in order to ascertain whether any residual effect of the iodine applied in 1931 could be detected. No consistent effect could be observed in the growth and yield of either beans or turnips, both of which produced considerably smaller yields than in 1931. A number of these samples have been analyzed, and no residual effects of the potassium iodide applied in the previous season have been found.

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SUMMARY

No relation was observed between the iodine content of potatoes, tomatoes, and sweet corn grown on field plots in a long-continued fertilizer experiment, and either the fertilizer treatment, the soil acidity, or the yield of the crop. The fertilizers used in the experiment were Chilean nitrate of soda, superphosphate, and muriate of potash.

An application of potassium iodide at the rate of 2.356 kg to the acre, supplying approximately 2.0 mg of iodine to the kilogram of soil in the upper 6 inches, increased the iodine content of green beans considerably as compared with that of beans similarly grown but without iodine fertilization, but it injured the plants and reduced the yields markedly. Similar applications of potassium iodide increased the yields of turnips somewhat, and increased the iodine content of the turnip plants from tenfold to 120-fold, on different plots. The greatest increases in iodine content of turnips occurred on plots made alkaline with hydrated lime, and the smallest increases on plots made acid with sulphur or unmodified in acidity.

The iodine content of turnips from plots not fertilized with potassium iodide was generally greater the more acid the soil; the iodine content of beans not treated with potassium iodide, however, was not influenced consistently by soil acidity.

The maximum recovery of added iodine by any turnip plants in the experiments was 11.4 percent. Analyses of plants grown on the same plots in the second season, without additional iodine, indicated that there was no residual effect of the iodine application.

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